SALTON SEA BIOLOGICAL REMEDIATION PROGRAM

Use of The Controlled Eutrophication Process (CEP) to Reduce Nitrogen and Phosphorus Concentrations Entering the Salton Sea

Final Report

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Prepared by
James M. Carlberg, Jon C. Van Olst, Michael J. Massingill,
Rodney J. Chamberlain, and Gregory Schwartz (Kent SeaTech Corporation)
David E. Brune (Clemson University) and John R. Benemann (Walnut Creek, CA)

Kent SeaTech Corporation
11125 Flintkote Avenue, San Diego, CA 92121

EXECUTIVE SUMMARY

Kent SeaTech Corporation conducts extensive research and development in water treatment and aquatic biology. Dr. David E. Brune and his colleagues in the Department of Agricultural and Biological Engineering at Clemson University are recognized worldwide as outstanding biological engineers who have developed successful solutions for a variety of difficult wastewater management problems over the past 30 years. Dr. John Benemann is a recognized expert in microalgae technologies. This partnership of leading private and academic researchers conducted a pilot-scale demonstration of the application of high rate algal pond water treatment technology to reduce the level of phosphorus and nitrogen nutrients that flow into the Salton Sea through its tributaries and cause serious eutrophication problems. This technology is termed the Controlled Eutrophication Process (CEP) and was originally developed as a cost-effective and environmentally sound technology for treating the low to medium concentrations of waste nutrients present in aquaculture effluent. Nutrient levels in the Salton Sea tributaries are similar to the levels found in aquaculture effluent and the CEP technology appears to provide a more cost-effective solution than either chemical treatment alone or the expensive Biological Nutrient Reduction (BNR) processes used to treat high strength municipal or industrial waste streams.

The project was conducted using existing pilot-scale CEP units that were available at Kent SeaTech's facilities in Mecca, CA, adjacent to the Whitewater River. All of the tanks, treatment system components, and data monitoring recorders required for this assessment were already in place and therefore the studies were conducted for a fraction of what the true cost would have been. A centrifugal pump and piping was installed on the Whitewater River to deliver river water to the test facility located just 100 meters away. The overall approach of the CEP concept is to stimulate rapid growth of algae in a well-mixed, high rate algal pond using a process design that permits accurate control of pond mixing rates, algal cell age, and nutrient concentrations. Nutrients are assimilated into the algal population and then removed from the ponds using gravity settling and consumption by managed populations of filter-feeding fish species such as tilapia. Studies were conducted to determine the optimal water flow rates, recirculation

strategies, filter-feeder densities, and other critical operating parameters so that the uptake and removal of phosphorus could be maximized. Related pilot-scale studies to refine techniques for settling, concentrating, and removing algal cells from the water column were conducted in existing systems located at Clemson University.

The results of these studies were very encouraging. The CEP process consists of two major treatment steps: 1) the assimilation of phosphorus and nitrogen into algal biomass, and 2) the removal or harvest of the algal biomass from the water column. In indoor, lab-scale tests, we were able to achieve nearly 100% efficiency for both of these steps. In the pilot-scale studies conducted in the 0.7 acre CEP units, we were able to achieve assimilation efficiencies as high as 83%, and algal removal efficiencies as high as 93%. The product of these two efficiencies, 77%, is the maximum overall treatment efficiency achieved during these trials, which were conducted in existing CEP units that were not originally designed for this application. We anticipate that even higher treatment rates can be achieved in CEP systems that are designed specifically for treating nutrients present in the Salton Sea tributaries.

 A techno-economic analysis was developed to estimate the capital and operating costs that would be required in a Demonstration-Scale application of CEP technology for nutrient bioremediation of the Salton Sea tributaries. The ultimate full-scale implementation of this concept would consist of a series of high rate algal ponds utilizing the CEP technology to reduce phosphorus and nitrogen in the Whitewater, New, and Alamo Rivers, which will significantly reduce the nutrient input driving the eutrophic conditions in the Salton Sea. Full-scale implementation of CEP technology for removing 80-90% of the eutrophying nutrient inputs to the sea is projected to require approximately 4,000 acres of land. In addition to filter-feeder biomass, the system would produce several valuable byproducts, including marketable fish, a concentrated algal sludge that could be used as a feed additive and as agriculture fertilizer, and energy from the on-site digestion of algae and production of methane.

The pilot-scale studies were very encouraging and are sumamrized in this report. The next step in developing this concept for application at the Salton Sea would be to construct and operate several full-scale CEP units of 10-20 acres in size, to obtain more accurate data on the operating costs and variability in treatment rates that will occur seasonally. Kent SeaTech is preparing several proposals to government agencies that would provide funding for these demonstration-scale studies.

BACKGROUND

The Salton Sea is a large inland lake (365 square miles) that is 227 feet below sea level, has no discharges, and has been accumulating salt and nutrients from stormwater, treated sewage, industrial waste discharges, and agricultural drainage for nearly 100 years. Over geologic time, the Salton Basin and Lake Cahuilla have been periodically flooded due to natural diversions of the Colorado River, but after each diversion ceased, the area became a dry lake bed. However, as the region developed and large-scale agricultural irrigation and municipal and industrial waste streams from the U.S. and Mexico were created, the Sea has become a permanent body of water, with evaporative losses approximately equal to tributary inflows. The buildup of salts and

nutrients from agricultural, municipal, and industrial discharges from the Imperial and Coachella Valleys has led to the development of an inland hypereutrophic, hypersaline lake.

Reduction of Nutrient Concentrations Entering the Salton Sea

Phytonutrients, in particular phosphorus and nitrogen, have reached excessive concentrations in the Salton Sea. The principal tributaries to the Sea (the Alamo, New, and Whitewater Rivers) contain 0.19 to 1.47 mg/L of total phosphorus and N/P ratios of 4.6 to 39.6 (CAL-99-001 RFP). Phosphorus is considered to be the limiting phytonutrient in the Sea. Although total phosphorus loading from the principal tributaries has doubled over the last 30 years (109%), the concentration in the Sea has not changed substantially (approximately 0.07 mg/L). Interestingly, the total nitrogen input loads have increased about 30% in the same time span, resulting in a concentration increase of about 50-60% (now about 5.0 to 5.4 mg/L). However, although the nutrient loading has increased, it has not significantly increased the eutrophic state of the Sea (Setmire et. al 2000).

The high algal growth potential of the Salton Sea caused by high levels of nutrients results in frequent wide-spread, high density algal blooms. Senescence of these blooms often leads to catastrophic low dissolved oxygen events, triggering massive and unacceptable fish mortalities and odor problems. A cost-effective solution to this problem will be difficult to achieve, since the volume of water to be treated is immense (more than one billion gallons per day). Also, if plans to create a smaller Sea are enacted, nutrient concentrations will be further increased.

There are several potential methods for reducing the input of nutrients into the Salton Sea and lowering the total phosphorus and nitrogen concentrations in the Sea itself. Chemical flocculation and sedimentation with metal salts such as alum could reduce the problem to some extent, although this method may be expensive. Although the loads of phosphorus entering the Sea are significant in relation to the eutrophication processes of the Sea, the average concentration of P in the rivers is quite low in comparison to the levels encountered in traditional wastewater treatment applications. The majority (54-87%) of the total phosphorus entering the Sea through the principal tributaries is present as ortho-phosphate and may not be easily precipitated using alum or other chemical flocculants in large-scale applications. Disposal of large quantities of alum-phosphate flocculant also may pose environmental problems. The Salton Sea Authority has funded a project by Dr. Chris Amrhein of UC riverside to investigate the effectiveness, costs, and potential problems of such a process.

The biological alternatives to chemical flocculation would involve concentrating the nutrients into some type of biomass and then removing this biomass from the tributaries and/or the Sea. Two methods of bioconcentration and conversion were discussed at the Salton Sea Eutrophication Workshop held in 2000: 1) wetlands conversion, which would involve largely bacterial processes, and 2) fish harvests, which would remove dead, moribund, or live tilapia from the Sea before they could release their biomass nutrients back into the water column or sediments. Wetlands generally store or remove nutrients seasonally and have been demonstrated to be effective at reducing BOD, suspended solids, ammonia (nitrification) and nitrate (denitrification) under specific conditions and loading rates.   Kent Seatech has operated about 90 acres of surface flow constructed wetlands (principally bulrush plant species) since 1996 as

part of its fish production water treatment and reuse system (24 MGD/day). Wetlands provide summer cooling effects, suspended solids reduction, nitrification, denitrification, and alkalinity control processes that permit significant recyling of water use in the production of hybrid striped bass. However, it is our belief that constructed wetlands have not been typically successful at significant phosphorus control in most demonstrated projects to date. The other alternative offered, harvesting of tilapia, could remove up to 10% of the annual external loading of phosphorus from the Sea, but "would have minimal impact on eutrophication" unless combined with other alternative solutions, according to Setmire et al.(2000).

Kent SeaTech and Clemson University have conducted extensive research, development, and demonstration involving a third concept for concentrating and removing nutrients from wastewater streams. The process is described as the Controlled Eutrophication Process (CEP), in which dense populations of single-celled algae are cultivated in high-rate algal ponds and then are removed (harvested) by several innovative methods prior to water flow exiting the system. The technology has been proven in a variety of applications in the U.S. and abroad. The Controlled Eutrophication Process represents the water treatment portions of the patented Partitioned Aquaculture System (PAS) developed at Clemson University during the past ten years. In joint research funded by the Department of Agriculture and the Department of Commerce Advanced Technology Program, Kent SeaTech and Clemson demonstrated the technical and economic feasibility of using CEP technology to reduce nutrient concentrations in aquaculture effluent. CEP technology can reduce the soluble nitrogen and phosphorus concentrations found in aquaculture effluent (7-15 mg/L and 0.5-2.0 mg/L, respectively) to very low levels. The levels of nitrogen and total phosphorus in the Salton Sea tributaries (8-18 mg/L and 0.7-1.1 mg/L) are similar to the levels present in aquaculture effluent and we believed that the CEP technology would perform similarly to our PAS process.

The Controlled Eutrophication Process

The basic concept of the Controlled Eutrophication Process (Figures 1-3, Photographs 1-6) is to utilize well-mixed, high rate algal ponds to grow dense populations of single-celled algae, which are maintained in a constant state of rapid growth. During this rapid growth phase, the algae are very efficient in assimilating dissolved nutrients in the surrounding water into their biomass. This initial step of the CEP process can be thought of as a conversion step, in which dissolved nutrients such as phosphorus and nitrogen are converted into particulate matter (algal cells). The next step is the harvest or removal of the algal cells from the water column. This is the more difficult step in the CEP process, since the individual algal cells are extremely small and have a specific gravity nearly identical to water. There have been many attempts to develop technologies for the harvest of single-celled algae from water, most often based on filtration, centrifugation, or settling concepts. However, most of these have proven to be inefficient and/or very expensive, so that they have only found commercial applications where the products involved made the harvest expense worthwhile.

The CEP technology for algal harvest is based on an entirely new concept. After exiting the high rate algal ponds (the Algal Treatment Zone), the water and algal cells enter the concrete channels (the Primary and Secondary Fish Zones), where appropriate filter-feeding fish such as tilapia are located. Fish in these Zones receive no other form of feed and will consume large quantities of

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single-celled algae. A portion of the algae that they consume is converted into fish biomass, and an even larger portion passes through the fish in their waste and is bound together in their fecal chains. The algal biomass is coalesced and bound by these processes into large particles that settle much more easily than individual algal cells. The concentrated and settled algal sludge is lifted up, dewatered, and transported out of the water column using an inclined belt harvest system that has been developed by engineers at Clemson University. A major advantage of this method of removal is that the end product is a thick algal slurry or concentrate that is high in nutrient content and can be used for a variety of fertilizer and biofuel applications.

The stoichiometry of algal biosynthesis in the CEP is observed to be similar to that reported by previous investigators (Shelef and Soeder 1980), with minor adjustments (from Meade 1998) in the C/N and C/O$_2$ ratios observed in CEP algal biomass production. This stoichiometry is:

$$106\,CO_2 + 16\,NH_4 + 52\,H_2O + PO^{-3} \rightarrow C_{106}\,H_{152}\,O_{53}\,N_{16}\,P + 106\,0_2 + 16\,H$$
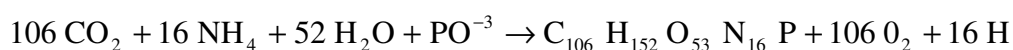
The observed CEP oxygen yield of 2.67 g of oxygen production per g of algal carbon fixation suggests a 1:1 oxygen to carbon molar ratio. In previous studies of CEP, the overall algal composition was observed to be approximately 50% carbon (by weight) at a C/N weight ratio of 5.7/1.0, and N/P weight ratio of 7.0/1.0. The N/P ratio of influent waters to the Salton Sea ranges from 4.6 to over 39:1. Algal processes are ideally suited for phosphorus removal under such conditions, especially in a location of high solar radiation such as the Coachella and Imperial valleys. Biological nutrient removal processes utilizing bacterial populations require a controlled energy input source to operate, either in the form of a correct BOD/P ratio (which is not likely in irrigation discharge waters) or an added external organic source. Sunlight is the driving force for the Controlled Eutrophication Process and the byproducts of treatment are medium to high value products (fish flesh, bioenergy and bio-available fertilizer concentrates).

Rationale for Selected Approach

The Controlled Eutrophication Process (CEP) is a form of Biological Nutrient Reduction (BNR) that is a very appropriate technology for this application, since it performs optimally at the low to medium nutrient concentrations that are found in the Salton Sea and its tributaries. In contrast, more intensive treatment technologies such as the Bardenpho BNR process and other bacteria-based methods used for treating municipal and industrial waste streams perform best at higher nutrient loadings, and in fact are generally inefficient at the lower concentrations such as those encountered in the Salton Sea and its tributaries. Although the limiting nutrient for the Sea is phosphorus, CEP technology removes both phosphorus and nitrogen eutrophying nutrients.

PROJECT OBJECTIVES

The overall objective of this project was to demonstrate the usefulness of the Controlled Eutrophication Process for high rate photosynthetic removal of nutrients from Salton Sea tributary waters. This was accomplished by addressing the following Tasks:

Task 1. Reconfigure two existing 0.7 acre CEP systems at Kent SeaTech to receive and treat Whitewater River water.

Task 2. Demonstrate that the CEP concept is capable of 90% removal of N and P by capturing the nutrient as algal biosolids and tilapia biomass.

Task 3. Determine optimum N/P ratio for algal growth, appropriate hydraulic retention times for each CEP Treatment Zone, and quantify flocculant requirements for Polishing Zone.

Task 4. Determine algal growth capacity, dominant algal species composition, and monitor uptake of selenium into algal and fish tissues.

Task 5. Evaluate the external carbon requirements of the system and filter feeder growth capacity.

Task 6. Evaluate the use of automatic algae removal belts for algal harvest.

Task 7. Quantify conversion of algal biosolids into tilapia biomass and N and P concentrate for use as agriculture fertilizer and biofuels.

Task 8. Utilize the resulting data to provide economic projections of the capital and operating costs involved in a full-scale implementation of the Controlled Eutrophication Process for the entire Salton Sea.

Task 9. Design a demonstration-scale CEP system for the Alamo River.

RESULTS

Preliminary Activities

A) Encroachment and Discharge Permits

The Coachella Valley Water District and the California Regional Water Quality Control Board (Colorado River Basin Region) have regulatory authority over the Whitewater River. It was necessary to request written permission from these agencies to install water intake and discharge systems in the river canal. The Coachella Valley Water District approved our request to install and operate an intake line to extract approximately 300 gpm of water from the Whitewater River (Encroachment Permit No. 07825-1-002). Slightly later, the California Regional Water Quality Control Board approved our application to modify our existing effluent discharge NPDES permit by adding approximately 300 gpm of discharge flow from the pilot-scale CEP treatment facility to the Whitewater River (Board Order No. 01-003).

B) Quality Assurance Project Plan

A Quality Assurance Project Plan (QAPP) was developed following the recommendations set forth in the EPA Quality Manual for Environmental Programs (U.S. EPA 2000). The QAPP follows the EPA recommendations to establish a Systematic Planning Process to set Data Quality Objectives (DQO's) for the CEP demonstration project. The QAPP also contains appropriate Decision Rules and Tolerable Limits on Decision Errors and an optimized design for obtaining useful results, including methods for sampling, handling, and analyzing the inflow and outflow streams. The QAPP was developed following suggestions offered by Dr. Barry H. Gump in the Chemistry Department of Fresno State University, who serves as the SSA QA Coordinator. Dr. Gump approved the QAPP that was submitted for the CEP project.

Task 1. Reconfigure two existing 0.7 acre CEP systems at Kent SeaTech to receive and treat Whitewater River water.

The CEP systems at Kent SeaTech are located near Avenue 70 in Mecca, CA, within 100 meters of the Whitewater River channel. Each CEP system measures approximately 90 x 30 meters, utilizing a single loop "U" design to minimize energy loss from turns (Photograph 4). The water depth can be adjusted up to a maximum of 1.2 m and the channel widths are 14.0 m. The Treatment Zone of each unit is constructed with outer earthen berms. The dividing channel walls are constructed of formed concrete. Fish are confined in screened raceway sections within the Fish Zone, which are made with rigid concrete walls to permit ease of harvest and fish grading operations. Water flow through the large Treatment Zones and also through the smaller Fish Zones is supplied by separate, slow rpm, variable-speed, hydraulically-driven paddlewheels (Photograph 2), capable of providing water velocities of up to 0.5 m/sec. Continuous paddlewheel aeration, emergency oxygen, and electronic oxygen monitoring systems are available in both CEP systems. U-tube aerators are supplied with gasified liquid oxygen to provide supplemental oxygenation in case of power failure.

It was necessary to modify the CEP systems for this pilot-scale demonstration, in order to provide zones for optimized algal growth (Algal Treatment Zone), initial algae harvest by tilapia (Primary Fish Zone), and final polishing by tilapia (Secondary Fish Zone). As described in the following sections, reconfiguration of the two CEP units was accomplished during the first three months of the project.

A) Modifications to Algal Treatment Zone

The Algal Treatment Zone and the fish holding compartments were completely dewatered to facilitate the required modifications. The CEP pond bottoms were scraped with a bulldozer in order to remove any organic matter that might have accumulated in the pond zones (Photograph 3). This was done since decomposition of residual organic matter could have influenced the levels of nitrogen and phosphorus measured in the CEP systems. The sides of the pond zones also were reworked, to repair some minor erosion. Construction contractors that are employed fulltime at Kent SeaTech completed these modifications.

B) Modifications to Fish Zones

 The fish raceway area used to hold the primary fish species during the previous aquaculture effluent pilot studies conducted in these units was modified in order to provide more area for algavorous fish to consume the algae and remove it from the water column. The concrete fish holding zones were reconfigured by removing several of the old cast concrete walls as required and installing reinforced concrete block retaining walls in new locations (Photograph 5). This same technique was used to construct the required support walls to permit the installation of the algal harvest conveyor belt designed by engineers at Clemson University (Photograph 6).

C) Design and Installation of Algal Sedimentation Belt

Studies were conducted at Clemson University to fine-tune the design of the Algal Sedimentation Belt for this pilot-scale evaluation (Photographs 25-30). Optimal water flow patterns for the belt settling zone were determined using three prototype algal harvest belts that were installed in 1/3 acre CEP test units at Clemson. Some initial problems were encountered due to the high mass of solids being removed by the belt, but these were rectified and the design for the belt for this project was finalized.

Composi-Tech Filters in Texas was selected to fabricate the large filter press conveyor belt required for the algal removal system. The dimensions of the continuous belt are 7.5 feet wide by 200 feet long. The belt is supported by a 3 inch angle iron track frame with cross supports every ten feet (Photograph 13). This support framework and track was fastened to the vertical walls of the concrete fish zone with concrete anchors. A center support of one inch steel flat stock runs down the center of the frame for added support. At one end, the belt is angled up and out of the water surface at an incline of 11 degrees, in order to lift the settled algal paste out of the chamber, dewater it, and divert it into a holding basin (Photograph 14). High-density nylon cleats are spaced every 9 inches transversely along the entire belt to prevent the algal paste from sliding down the inclined portion of the belt. A roller and gearmotor at the upper end of the belt

cause it to advance at a speed of 0.7 feet per minute. This speed was determined to be slow enough to not resuspend the settled paste and not create noticeable turbulence.

In a series of tests, we determined that the Algal Sedimentation Belt worked very well to gently remove the settled algal biomass from beneath the Primary Fish Zone of the CEP system. We found that it was advantageous to operate the belt intermittently rather than continuously, since the settled algae appeared to agglomerate and form a thicker paste when it was left undisturbed for longer periods (Photograph 21-24).

D) Construction of Fish Cages

Although the initial design for this system involved the confinement of the filter-feeding fish in net-walled compartments, we determined during our preliminary evaluations that a better design would be to confine the fish in rigid-walled steel cages with perforated bottoms. The advantage of this design is that the fish do not have access to the surface of the belt at the bottom of the compartment and thus cannot disturb the settled algal biomass. Also, stocking, harvesting, and moving of the populations of fish is greatly facilitated by having them in cages, which are easily lifted and moved from zone to zone as needed, using a small crane or backhoe. The fish cages were constructed of expanded steel screen that is welded to a 1.5" angle iron frame. The size of the fish cages is 7.5 ft by 8 ft. by 2.5 ft deep (Photograph 19).

E) Installation of Flocculator

In order to evaluate the use of chemical flocculants that might be needed during the colder months as a final polishing step in the CEP process, a small flocculator was constructed and mounted inside the fish raceway chamber over the belt. The flocculator consists of two compartments (Photograph 17). The flocculant is introduced into the flash mixing chamber, designed for a one minute contact time with a water flow rate of 40 gpm. The mixture then flows into the slow mixing chamber, consisting of a chamber with strategically placed baffles, which was designed to provide a 10 minute contact time. This chamber allows the simultaneous build-up of flocculated particles to occur, while gradually decreasing the mixing energy to prevent break-up of the associated particles. The flocculated agglomeration is then allowed to settle onto the Algal Sedimentation Belt during a five hour detention/settling time.

F) Whitewater River Intake and Discharge System

The Whitewater River is located approximately 500 meters from the CEP units at our facility in Mecca, CA, and has large flood control earthen levees on either side to prevent flooding of surrounding farmland during winter storms and flash floods (Photograph 7). It was necessary to install PVC intake and discharge piping under the west levee, which might have been an expensive and time-consuming project, and would have required several permit delays. Fortunately, Kent SeaTech had already installed a large 27 inch diameter drainage pipe under the west levee in a location that was fairly close to the CEP units. We decided to use this single large pipe for both the input and discharge functions by inserting an 8 inch PVC intake line inside the existing 27 inch discharge pipe (Photograph 8). 20 ft sections of 8 inch PVC schedule 40 pipe were inserted into the east end of the drainage pipe at the point where it reaches the

River bank. The ends of the 20 ft sections were solvent welded and then the entire length of 8 inch pipe was pushed slowly into the drainage pipe using a backhoe. A foot valve was placed at the end of the intake piping in the Whitewater River. The intake was installed at an angle so that it is located upstream from the discharge flow from the 27 inch pipe.

Shortly after we began testing the system, we determined that there was considerable debris in the River canal and therefore we constructed a 4 ft x 4 ft x 4 ft screen cage of expanded steel screen in order to protect the foot valve from fouling. This solution worked well until midsummer of 2003, when the CVWD decided to dredge the Whitewater River for periodic storm management. During this period, the foot valve clogged frequently and for several weeks it was necessary to operate the CEP units on an alternative water supply, which had similar levels of phosphorus and nitrogen.

At the west end of the intake pipe, a service pit was constructed so that the piping could be brought up to grade level. At this point a 220V 3 phase electrical service was installed and two 3.0 hp centrifugal pumps were installed on a concrete pad (Photograph 9). Each of the pumps is capable of providing approximately 200 gpm of flow through 8 inch pipe to the CEP units (Photographs 10 and 11). A large volute pump design was selected to reduce the effects of centrifugal pumping on the algal populations present in the river water and to more closely simulate a full-scale application that would utilize low-head lift pumps and/or gravity flow to deliver the river water to the CEP system. A pipe manifold also was installed at the pump pad, consisting of suction-side valves, pump-side valves, and check valves. A two inch priming valve also was added, to be able to prime the pumps as required. Discharge from the CEP units is handled through a drainage standpipe system that was in place from previous studies.

G) Installation of Filter-Feeder Evaluation Tanks

A multi-tank system was needed for preliminary filtration trials to facilitate the design process and permit us to rapidly develop data on the filter feeding rates of various size classes of algavorous fish, using algae grown in water taken directly from the Whitewater River. A concrete pad was poured adjacent to the CEP units to serve as a base for a system of six circular experimental tanks (Photograph 12). Six 350-gallon polyethylene tanks and four 150-gallon fiberglass troughs were provided by Kent SeaTech. A water supply tank was placed 4.0 ft higher than the experimental tanks to allow for steady input flow by gravity. The circular tanks were supplied with Whitewater River water and with water from the pond zone of the CEP system, to allow us to conduct short range-finding studies on the algae removal rates of the algavorous fish used to consume algae in the CEP units. These tanks were plumbed to allow operation as two batch reactor systems and four continuous flow reactor systems, which allowed us to evaluate various operational configurations before implementing the test conditions in the larger CEP units. Fish holding compartments were installed for convenient periodic samples and surveys. Oxygen lines were plumbed to the system to meet the respiratory requirements of the fish during the experiments. Electrical conduit and wiring were installed to supply the water pump and the control system for the automatic batch sequencing valves.

H) Carbon Dioxide System

VGL cryogenic tanks filled with liquid carbon dioxide were used to supply CO_2 to the CEP systems to provide the necessary carbon for algae growth. A concrete slab was poured to support the heavy VGL systems, which were delivered periodically by an outside contractor. The CO_2 gas was piped into an aeration U-tube in order to mix it and dissolve it prior to delivery to the algal population.

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Task 2. Demonstrate that the CEP concept is capable of 90% removal of N and P by capturing the nutrient as algal biosolids and tilapia biomass.

After completing the required modifications of the CEP units, the pumps supplying water from the Whitewater River were turned on and the units were filled. During the first few days of operation, we adjusted the paddlewheels that control the water flow and velocity. After this, we began to condition and acclimate the CEP units in preparation for a 12 month evaluation period to determine the optimal conditions of operation for removing phosphorus from the Whitewater River. We expected that seasonal differences in temperature, sunlight, nutrient loading, and algal population changes would result in significant changes in the treatment ability of the CEP system during the year. The following sections describe our activities during the one-year evaluation and the results of water quality observations of the CEP system under several different modes of operation and nutrient loading rates.

A) Stocking of Filter-Feeding Fish

At the beginning of the acclimation period, we stocked populations of juvenile tilapia that were being held in circular tanks into the Primary Fish Zone of the CEP system, at an initial density of about 225 juveniles/m^3. During the pilot-scale evaluation, monthly subsamples of these populations were taken to provide estimates of the growth, survival, and health of the algavore species. The biomass of the fish population was increased or decreased as necessary periodically during the evaluation to determine the optimal balance between the rate of algae harvest and the density of the algae in the Algal Treatment Zone. Additions or subtractions of tilapia biomass occurred in October 2002, May 2003, July 2003, and August 2003. Each time, the average weight of the fish and their total biomass was recorded. During the summer of 2003, we observed considerable bird predation on the fish and at that time we constructed mesh lids for the fish cages to prevent bird entry.   The results of our studies of filter-feeders are described in Tasks 4, 5, and 7 below.

B) Acclimation of CEP System

The CEP units were provided with a continuous flow of up to 100 gpm/ac of water from the Whitewater River starting in the second week of August 2002, and allowed to acclimate for a period that was expected to take approximately 60 days. Preliminary water velocity studies were conducted during this time to make sure that the flow through the Treatment Zone was uniform and provided a current velocity that would keep the algae populations in suspension. During the acclimation period, the population of algae rose and fell in short blooms and crashes, as can be seen from the wide variations in the Secchi Disk Visibility readings for September and October of 2002 (Figure 4). By the end of the third week of acclimation, a consistent and sustainable algal bloom still had not been established. In previous CEP studies at Clemson University, sustainable algal blooms in new ponds sometimes took as long as two months to develop. Under these conditions, soluble phosphorus fertilizer sometimes was used to help stabilize the algal bloom.

Figure 4. Secchi Disk Visibility (SDV) for CEP Unit 2 during the pilot-scale
studies. The SDV readings correlate very well with measurements of TSS and
VSS, which are directly correlated with the amount of algae present in the water.
The SDV readings could be taken daily and at very low cost.
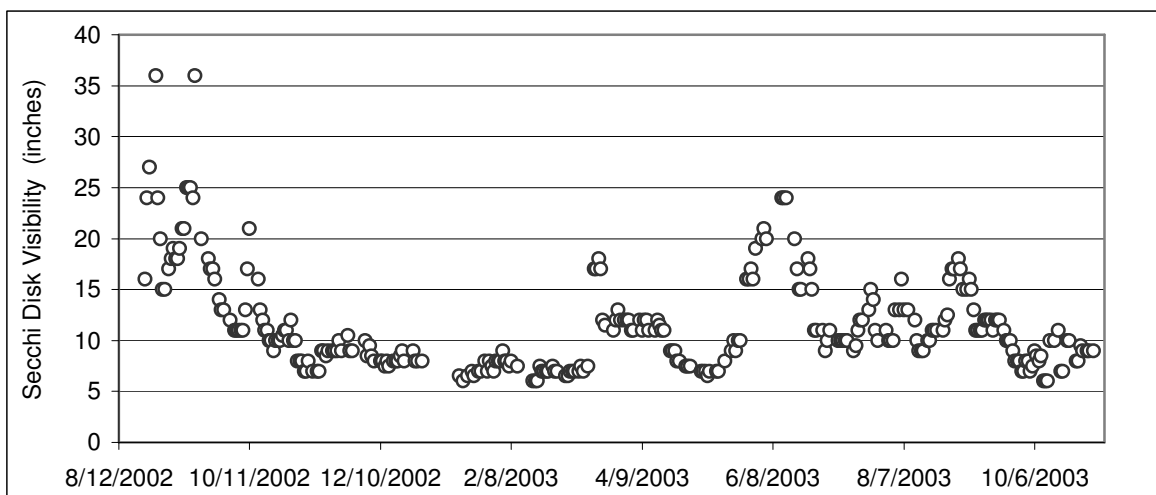
Several more bloom and crash cycles were experienced before a sustainable algae bloom was
achieved in November 2002 (Figure 4). During these rapid fluctuations in the algal population,
the SDV readings decreased to about 15 inches as the population bloomed and then a subsequent
crash would occur and the SDV would increase to about 35 inches in just a few days. A second
crash occurred shortly after the first, with SDV readings of 15 inches increasing rapidly to 35
inches. After the second crash, soluble phosphorus fertilizer was added to stabilize the algal
biomass. A third, smaller crash occurred in the middle of October, increasing the SDV from 10
inches to 20 inches, after which the SDV was stabilized. Considering that the previous Clemson
experience has been with closed system CEP units, whereas the system installed on the
Whitewater River is a flow-through, open system, a 90 day acclimation period appears
reasonable.

Due to the initial difficulties in sustaining an algal bloom, there was some concern that
unexpected toxicants might be present in the Whitewater River that were affecting the planktonic
algae. For this reason, a laboratory-scale algal growth assay was performed. Whitewater River
water samples along with control samples taken from a groundwater well were used in the
laboratory to culture algae under optimal conditions. In this setting, a stable algal bloom was
achieved in approximately 15 days, and within 60 days, the algae was removing 100% of the
soluble phosphorus and 80 – 90% of the soluble nitrogen as shown in Table 1. The result of the
laboratory assays indicated that the Whitewater River water could easily sustain a dense algae
bloom. For additional verification regarding the potential for toxicity, we also sent two
Whitewater River water samples to an independent laboratory (Aquatic Testing Laboratories of
Ventura, CA) for *Selenastrum* algal growth chronic toxicity testing (EPA method 1003). Neither
sample was significantly different than controls, using a Homoscedastic t-Test at p=0.05.
Shortly after these tests, a stable bloom was established in the CEP system.

Table 1. Laboratory-scale studies of growth of algae in Whitewater River water, as indicated by the ability of the algal population to uptake nitrogen and phosphorus nutrients.

Soluble N Added (mg/L)	Soluble N Concentration (mg/L)	Soluble N Removed (mg/L)	Soluble P Added (mg/L)	Soluble P Concentration (mg/L)	Soluble P Removed (mg/L)	% Soluble N Converted to Algae	% Soluble P Converted to Algae
7.7	1.1	6.6	1.2	0.0	1.2	86%	100%
7.5	0.8	6.7	1.1	0.0	1.1	89%	100%
6.7	2.6	4.1	0.9	0.0	0.9	61%	100%
5.9	0.5	5.4	0.7	0.0	0.7	92%	100%
5.7	0.7	5.0	0.7	0.0	0.7	88%	100%
7.2	0.6	6.6	1.1	0.0	1.1	92%	100%

C) Water Velocity Profiles

Successful mass culture of single-cell algae in a large open pond system requires a well-mixed water column with uniform flow patterns and few dead zones. A well-mixed water column helps to keep the algal biomass in suspension, while uniform flow patterns help to keep the algal cells growing at a uniform rate. Keeping the water column well-mixed also promotes maximum growth rates from the algae by constantly turning the water column over for solar radiation exposure. Mixing in the Algal Treatment Zone of the CEP system is accomplished using highly-efficient paddlewheels that are able to move very large volumes of water and yet consume only a few horsepower. In order to determine the water velocity profiles in the CEP units, flow velocity, direction, and depth information was recorded. The data indicated that flow was fairly constant in most sections of the CEP units, but there were a few areas of low flow at the far end of the dividing wall (Figure 5). In a full-scale implementation of CEP, the ends of the Algal Treatment Zones would have semi-circular flow dividers to uniformly channel the flow around the 180°bends, which will reduce or eliminate the areas of uneven flow.

Figure 5. Water velocity, depth, and flow profile measured in the Algal Treatment Zone of CEP unit 2. Pairs of numbers indicate water depth in inches followed by velocity in ft /sec. Numbers in circles indicate total flow (cfm) calculated from the sum of velocity-depth profiles at each measuring station. Arrows indicate approximate direction of flow.
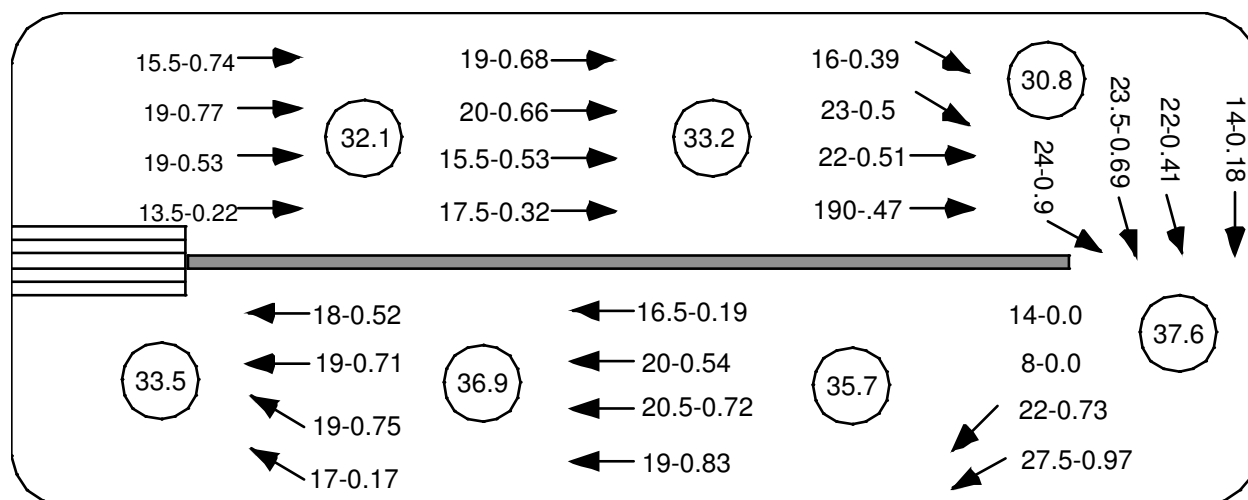
D) Water Quality Analyses

Water quality analyses were conducted throughout the study to better determine the performance of the CEP systems under a variety of operating conditions. In order to achieve maximum removal of nutrients, the two most important factors are the algal growth rate and the algal standing crop. The daily mass of nutrient removal in the CEP is dependent on the product of the individual algal growth rate and the algal standing crop. A direct measure of productivity is obtained by measuring photosynthesis and respiration using light/dark bottles, which will indicate the carbon fixation rate of the algae (discussed in Task 4). The algal growth rate, or productivity, can be estimated by measuring daily diurnal pH and oxygen concentration shifts. The algal standing crop is the biomass of algae in the water column at any given time. A convenient parameter that can be measured instantly and used to estimate algal standing crop is Secchi Disk Visibility (SDV). A more direct measure of the algal standing crop can be obtained by measuring volatile suspended solids (VSS). These and other pertinent water quality factors measured in the CEP systems are discussed below.

1) Basic Water Quality Data

pH is a useful indicator of algal productivity because photosynthesis raises the pH and algal respiration decreases the pH. For systems with rapid algal growth rates, the daily pH fluctuation may range from 7.5 to 9.5 under optimal conditions without the addition of an external carbon source. To prevent carbon limitation in this application of CEP technology, external carbon must be supplied. The addition of carbon dioxide results in the formation of carbonic acid and lowers the pH values. The optimal range for pH when CO_2 is being supplemented in high-rate algal pond systems is between 7.0 and 8.0. The challenge in maintaining a low pH is that when the algal growth rate is high, the rate of CO_2 addition must also be high in order to maintain pH at 8.0 or less. Also, the total daily requirement of CO_2 needs to be delivered in a short (4 to 6 hour) time period, greatly influencing the method of delivery.

pH and water temperature were measured daily and are shown in Figures 6 and 7. Monthly average morning and afternoon pH data for the previous 12 months are shown in Figure 6, along with the average daily amount of carbon dioxide that was added. The pH values shown for August through October, 2002, represent data taken during the initial acclimation period (a period of low productivity) when little photosynthesis was occurring and therefore the average afternoon pH was relatively low. As the algal biomass increased, so did photosynthesis and the afternoon pH. January through March was a period of high algal biomass and good productivity and as a result, the maximum pH rose to above 9.0. In March, carbon dioxide applications were increased, thereby reducing both the morning and afternoon pH values. For the month of June, carbon dioxide additions were not required due to the low productivity caused by increased algal grazing by high densities of filter-feeding fish that were stocked intentionally during studies of the effects of fish density on grazing rates.

Figure 6. Average monthly variation in morning and afternoon pH values recorded in the CEP system, and the average amount of carbon dioxide that was added daily. CO_2 was added as a carbon source for algal growth and also served to reduce the high afternoon pH levels.
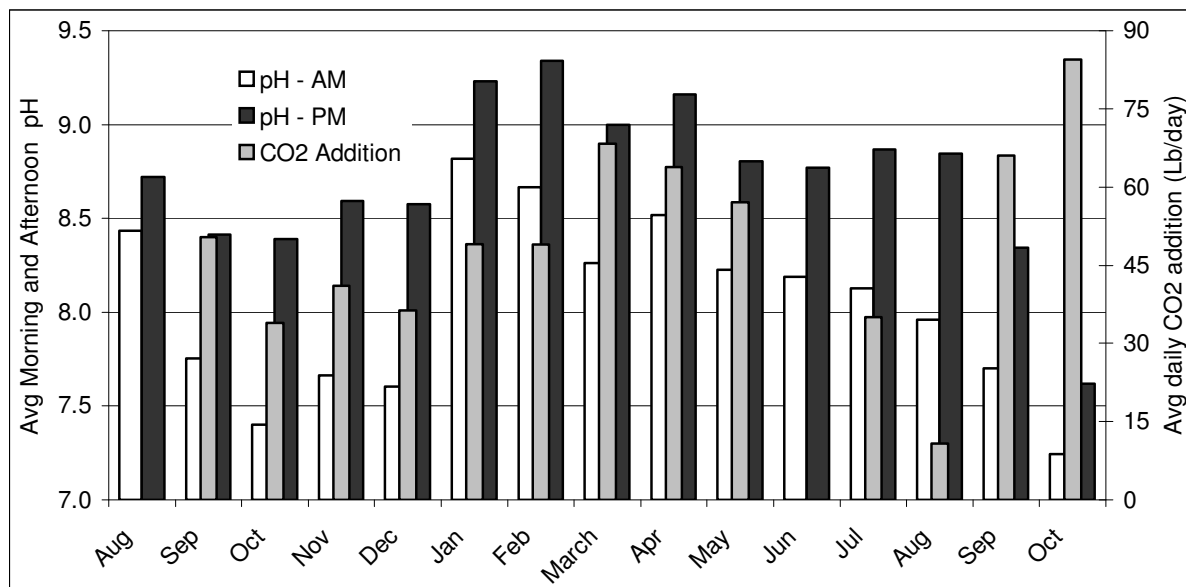
Temperature was measured twice daily to record the morning and afternoon temperatures (Figure 7). During the year, the temperatures recorded in the CEP system ranged from a minimum of 10°C to a maximum of 35°C. The algal growth rate in the CEP system is partly a function of temperature. Maximum theoretical productivities are several times higher in summer than in winter, due to a combination of annual variations in temperature and annual variations in solar radiation caused by sun angle and cloud cover. These seasonal changes result in a higher rate of nutrient removal during the warmer months.

Figure 7. Morning and afternoon water temperatures recorded in CEP system.
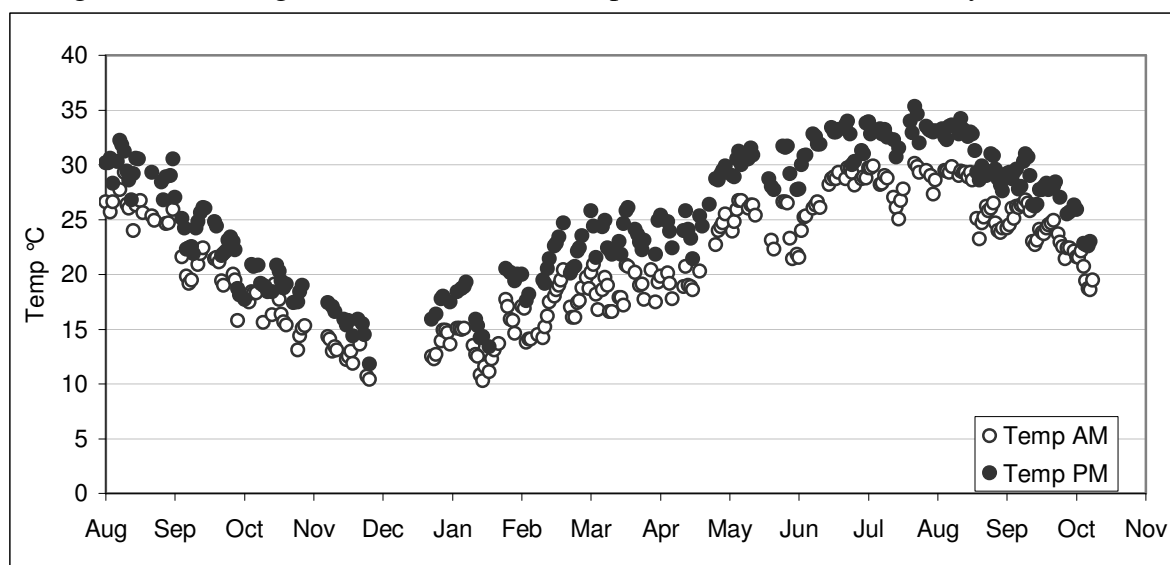
2) Algal Density (Secchi Disk Visibility, TSS, VSS)

Total suspended solids (TSS), volatile suspended solids (VSS), and Secchi Disk Visibility (SDV) were measured frequently in the CEP system (Figure 8). TSS and VSS increased soon after the acclimation period began. Several decreases in TSS can be observed over the course of operation of the unit, which are attributable to reductions in the algae population that occurred while we varied the water inflow rates, the recirculation rates, and other factors as we learned to operate the system for optimal removal of phosphorus. The largest reduction occurred in June 2003, when we tested the highest stocking density of filter-feeding fish in the Primary Fish Zone. These fish were able to consume the algae extremely rapidly. These are very encouraging findings, since they indicate that significantly fewer fish will be needed to manage the algal population in a full-scale CEP application. In July 2003, we reduced the fish population and the algal population rebounded rapidly.

Figure 8. Total suspended solids (TSS), volatile suspended solids (VSS), and Secchi Disk Visibility (SDV) measured in the CEP system.
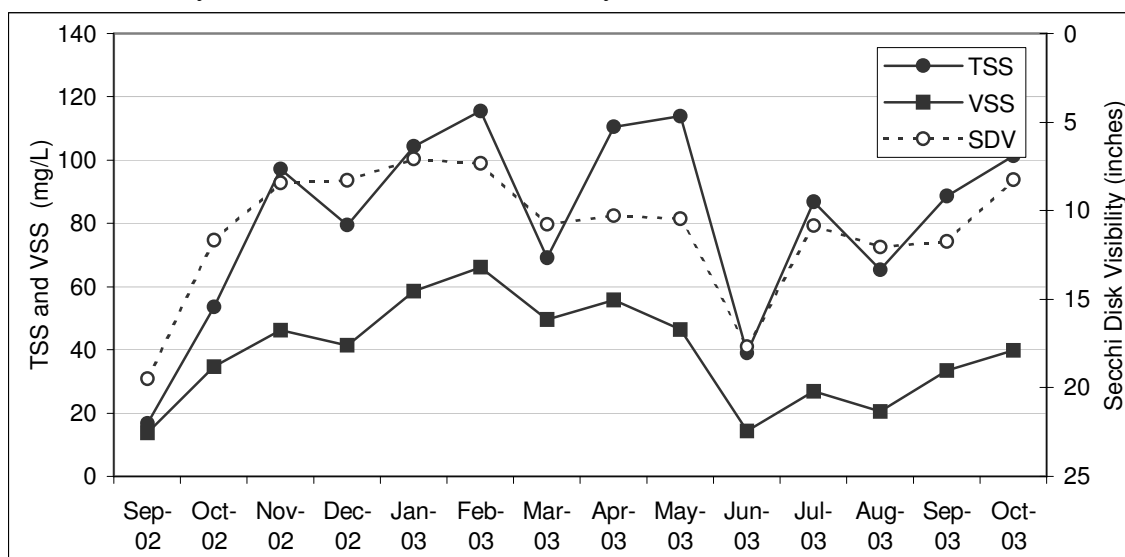

Secchi Disk Visibility (SDV) was measured daily. SDV is a rapid and reasonably reliable estimate of the algal standing biomass. Measuring volatile suspended solids (VSS) provides a more exact measure of algal biomass, but is more expensive and time-consuming. As seen in Figure 8, changes in SDV closely followed the changes observed in TSS and VSS. As SDV reached six inches in January, TSS and VSS peaked at 118 and 65 mg/L, respectively. When the algal population crashed in March due to changing temperatures and shifting algal species, TSS and VSS dropped to about 70 and 50 mg/L respectively, and SDV increased to 10 inches. During the period of high algal harvest caused by the high levels of filter-feeders stocked in June, TSS and VSS dropped to 40 and 18 mg/L and SDV increased to 18 inches.

As shown in Figure 9, there is a fairly close inverse exponential relationship between SDV and VSS (r^2=.74). SDV can be influenced by other suspended solids in the water column, but in practice, it can be used as an inexpensive and reliable technique to monitor and manage algal ponds.

Figure 9. Exponential relationship between SDV (inches) and VSS (mg/L)
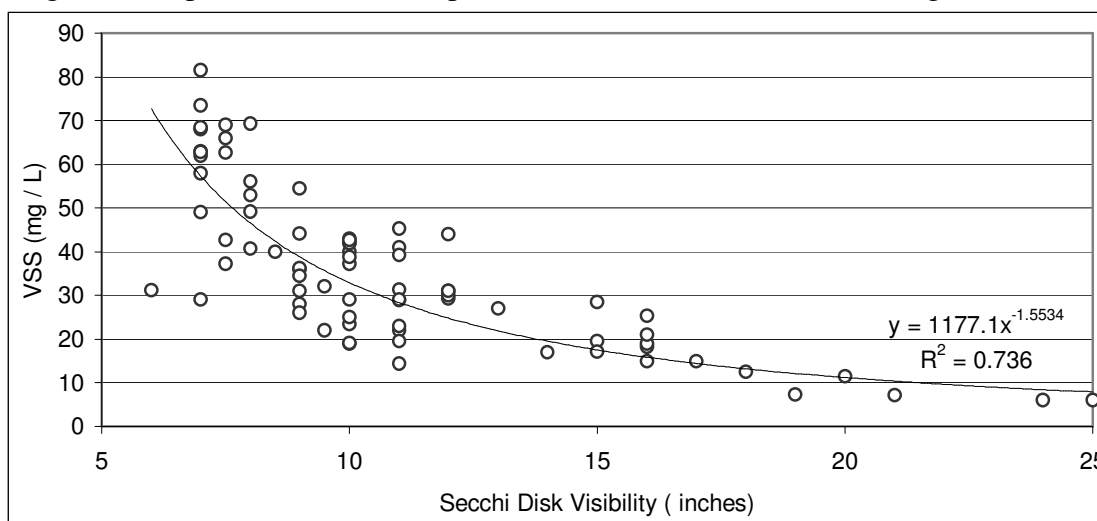
Examination of the daily SDV values in Figure 10 indicates how useful these measurements can be in documenting rapid changes in the algal population, such as the exact day of the algal crash in March (3/18) or the period in which the high stocking levels of tilapia began to result in overharvest (5/20). As indicated in these data, once an algal bloom has been stabilized, even after crashes the algae population tends to quickly bounce back.

Figure 10. Variations in Secchi Disk Visibility (SDV) observed in CEP Unit 2
after the acclimation period was completed.
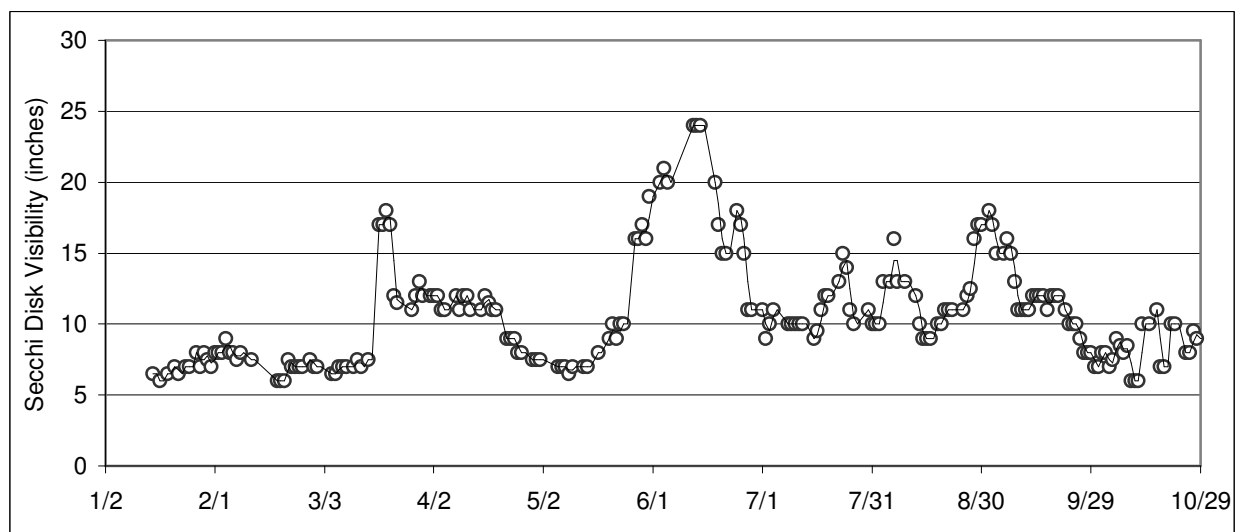
3) Nutrient Concentrations (TP, SP, TN, AN, NN, NiN)

Nutrient concentrations were measured in the Kent SeaTech Water Quality Laboratory, with periodic samples sent to a certified laboratory for outside verification. The independent tests agreed well with the results of our own analyses. Whitewater River water was the primary input water source to the CEP unit, although during the period of October through December 2002,

fish farm effluent water was used as the source water. Several experiments were run during this time period, in which we needed to obtain data on algal N:P ratios as a function of various system water column N:P ratios. Utilization of the fish farm water provided a source of water with a very low N:P ratio for these tests that the Whitewater River source water could not provide. Whitewater River water quality measurements began at the end of December.

Water quality data, consisting of measurements of total phosphorus, soluble (ortho) phosphorus, total nitrogen, nitrate nitrogen, nitrite nitrogen, and ammonia nitrogen, were made weekly for both the incoming water to the CEP unit and the CEP Algal Treatment Zone (Table 2). In addition, an aliquot of CEP water was centrifuged to produce samples of algal plug and decant (supernatant) water. The following figures summarize the water quality observed in the Whitewater River input water and in the CEP system throughout the project.

Total phosphorus concentrations in the Whitewater River remained relatively stable throughout the year. Slightly higher total phosphorus values were present during the winter months (approximately 1.7 mg TP/L). During the summer, total phosphorus decreased slightly to about 1.4 mg TP/L. As indicated in Figure 11, there were several brief spikes in the observed levels, which may be accounted for by periodic siltation problems in the intake structure so that the foot valve was nearly buried and the pump entrained large amounts of bottom silt. The soluble or ortho-phosphorus measured in the Whitewater River was consistently less than the total phosphorus by about 0.2 – 0.4 mg/L.

Figure 11. Weekly measurements of the total phosphorus concentration in the incoming Whitewater River water for the CEP units during 2003.
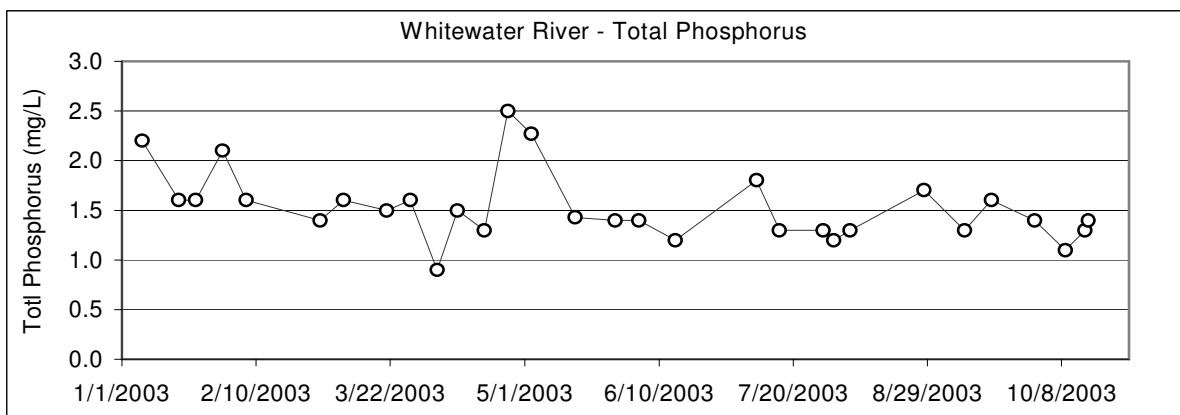
The amount of total phosphorus assimilation into algal biomass in the CEP was calculated by comparing the incoming water total phosphorus to that of the supernatant of centrifuged CEP algal channel water. The average assimilation of phosphorus into algal biomass for the winter, spring, summer, and fall of 2003 is shown in Figure 12. The seasonal average Whitewater River total phosphorus concentrations were 1.7, 1.6, 1.4 and 1.6 mg/L, respectively, while the supernatants after CEP treatment were 0.6, 0.5, 0.3 and 0.7 mg/L, respectively, resulting in 64%, 70%, 77% and 56%, respectively, of total phosphorus assimilation. The removal rates improved as we became more skilled in managing the algal population in the CEP system to achieve maximum algal growth, and should be even higher as more information is obtained.

Figure 12. Average seasonal assimilation of total phosphorus into CEP algal biomass.
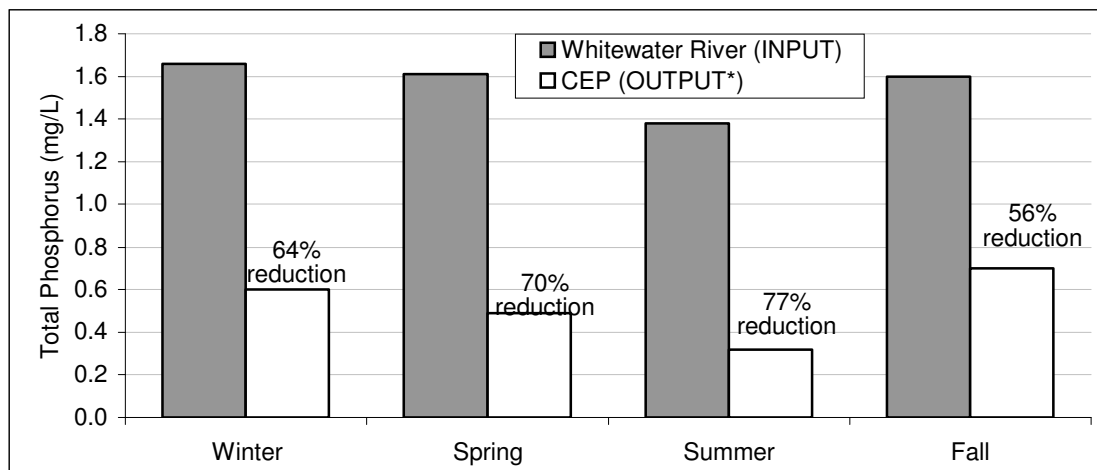
Using tilapia as the biological filter-feeder and the algal sedimentation belt, our studies indicate that 85-94% of the algal biomass can be harvested and removed. The product of the two efficiencies, algal assimilation of phosphorus and filter-feeder/belt removal of algae, results in the overall efficiency of the CEP process. (If required, additional polishing by flocculation would be added at the end of the treatment chain.)

Total nitrogen concentrations in the Whitewater River varied from a low of approximately 11 mg/L in April 2003 to a high of nearly 25 mg/L in July (Figure 13). One possible explanation for the fluctuations observed in annual total nitrogen levels is that agriculture fertilizer applications in the summer increase the nitrogen concentrations in agriculture drainage. As indicated in Table 2, the predominant form of nitrogen present is nitrate.

Figure 13. Weekly measurements of the total nitrogen concentration in the incoming Whitewater River water for the CEP units during 2003.
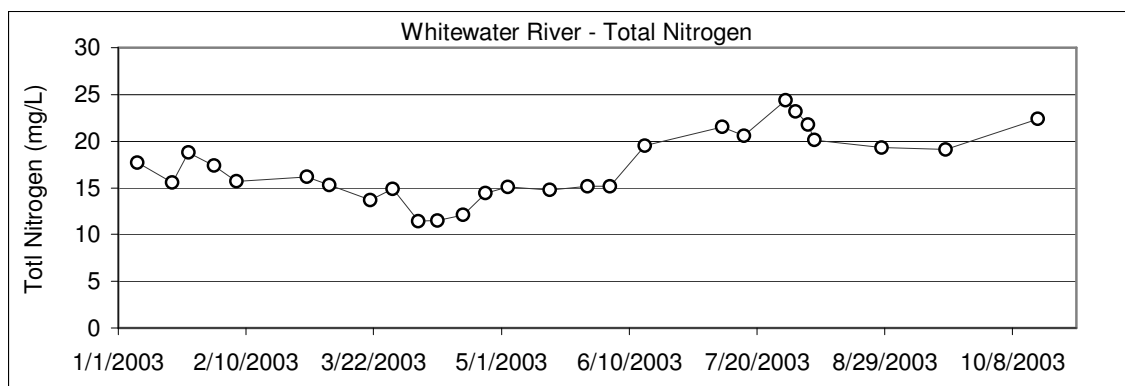
The amount of total nitrogen assimilation into algal biomass in the CEP was calculated by comparing the incoming water total nitrogen to that of the supernatant of centrifuged CEP algal channel water. The average assimilation of nitrogen into algal biomass for the winter, spring, summer, and fall of 2003 is shown in Figure 14. Incoming seasonal average nitrogen

concentrations were 17, 14, 22 and 19 mg/L, while the centrifuged supernatants were 12.5, 6.0, 10 and 11 mg/L, respectively, resulting in 26%, 57%, 55% and 43% total nitrogen removal. Similar to phosphorus assimilation, the percentage of nitrogen that was assimilated into the CEP algal biomass increased as the algal population stabilized and we became more familiar with the operating parameters of the system.

Figure 14. Average seasonal assimilation of total nitrogen into CEP algal biomass.
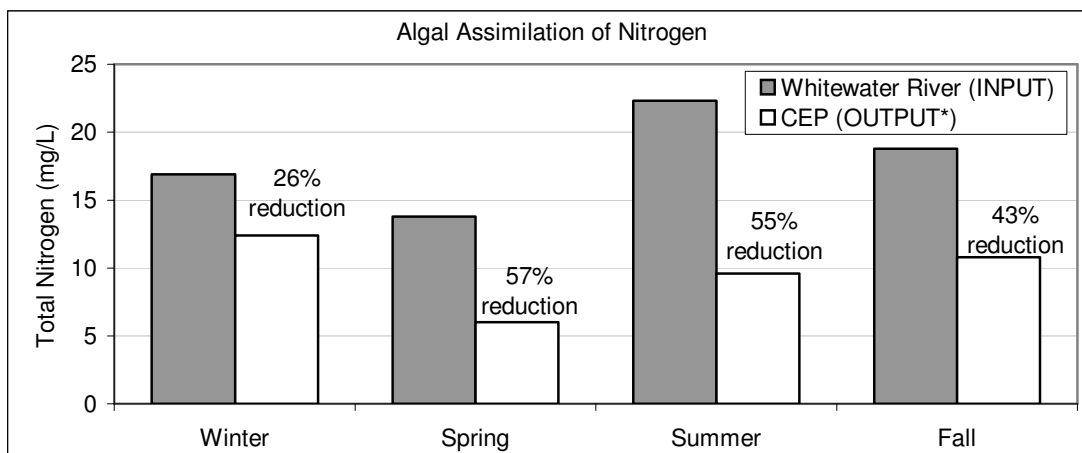

Table 2 provides the weekly water quality data from the Whitewater River in tabular form. Data are shown for both ortho phosphorus and total phosphorus. Ortho phosphorus represents the soluble portion of phosphorus entering the CEP units. The three different forms of nitrogen also are shown. Nitrate nitrogen is the dominant form of nitrogen, usually consisting of about 80% of the total nitrogen present in the Whitewater. Algae preferentially use ammonia as their nitrogen source and therefore any ammonia brought into the CEP from the Whitewater River flow is utilized almost immediately. Ammonia nitrogen usually made up only about 4 to 15% of the total nitrogen, depending on the season. The nitrite levels were very low in the incoming water and played no significant role as a nitrogen source for the algae.

E) Removal of Settled Algal Biomass

Phosphorus and nitrogen present in the Whitewater River input flow are converted to algal biomass in the CEP Algal Zone. The algal biomass can be removed from the system by three primary pathways: 1) unsettled algae can pass through the system and be discharged in the CEP effluent, 2) algae can settle in the Algal Zone and be bound in the bottom sediments, and 3) algal biomass can be concentrated in the CEP Fish Zones and settle on the Algal Harvest Belt. Our studies determined that most of the phosphorus was removed during the CEP treatment and did not pass through to the discharge, and that the Algal Harvest Belt was very efficient in removing algal biomass settled in the Fish Zones. It is possible that up to 1/3 of the phosphorus removal was the result of algal sedimentation or direct inorganic phosphorus precipitation in the Algal Zone. This result is similar to the levels observed in experimental trials at Clemson University, where in-pond settling is used as an inexpensive means of phosphorus removal. In follow-on studies, this mechanism will be investigated in greater detail as a possible alternative sink for phosphorus removal in CEP applications.

Table 2. Weekly measurements of TSS, VSS, phosphorus, and nitrogen present in the
Whitewater River supply water delivered to the CEP units.

| Date	TSS mg/L	VSS mg/L	NH3 mg/L	NO2 mg/L	NO3 mg/L	Total N mg/L	Ortho P mg/L	Total P mg/L
1/7/03	11.2	4.0	2.2	0.8	14.0	17.7	2.2	2.2
1/18/03	15.2	5.6	1.7	0.8	12.8	15.6	1.5	1.6
1/23/03	13.0	7.0	2.1	0.9	14.7	18.8	1.5	1.6
1/31/03			2.4	1.0	14.5	17.4	1.9	2.1
2/7/03	7.6	4.0	2.9	0.8	10.0	15.7	1.3	1.6
3/1/03	12.8	6.8	1.5	0.9	10.6	16.2	1.1	1.4
3/8/03	31.7	7.9	2.0	0.9	14.9	15.3	1.3	1.6
3/21/03	16.8	8.8	1.3	1.0	10.2	13.7	1.2	1.5
3/28/03	16.5	5.5	1.5	1.1	10.2	14.9	1.4	1.6
4/5/03	9.0	3.0	1.6	0.7	9.6	11.4	0.8	0.9
4/11/03	13.3	4.3	2.4	0.8	8.8	11.5	1.2	1.5
4/19/03	15.5	7.5	2.2	0.8	8.9	12.1	1.2	1.3
4/26/03	171.8	16.5	2.6	0.8	8.2	14.4	1.5	2.5
5/3/03	68.0	6.5	5.8	0.8	8.9	15.1	1.9	2.3
5/16/03	11.0	3.0	3.7	1.2	9.2	14.8	1.3	1.4
5/28/03	90.0	16.0	1.6	1.0	10.2	15.2	1.0	1.4
6/4/03	44.0	8.8	2.0	1.1	13.6	15.2	1.2	1.4
6/15/03	60.0	10.0	1.1		16.6	19.5	1.0	1.2
7/9/03	114.0	12.0	1.0		19.0	21.5	1.0	1.8
7/16/03	41.2	6.0	0.6		21.0	20.6	1.1	1.3
7/29/03	51.0	6.8	1.0		23.1	24.4	1.0	1.3
8/1/03	32.0	8.0	1.4		18.0	23.2	1.0	1.2
8/5/03	53.0	11.0	1.0		21.2	21.8	1.0	1.3
8/7/03	79	14	0.67		20.1	20.8	0.92	1.3
8/28/03			0.71		19.3	18.8	1.1	1.7
9/7/03								1.3
9/17/03	87	18	0.74		19.1	23	1.1	1.6
9/30/03								1.4
10/9/03								1.1
10/16/03			0.66		22.7	22.4	0.57	1.4

Task 3. Determine optimum N/P ratio for algal growth, appropriate hydraulic retention times for
each CEP Treatment Zone, and quantify flocculant requirements for Polishing Zone.

A) Nitrogen:Phosphorus Ratio

Efficient removal of phosphorus through the algal growth process requires that the N:P ratio of
the input water be maintained within optimal limits. If the ratio of nitrogen is too low,
inadequate algal growth will result. During this project we evaluated the possibility that the N:P
ratio of the influent to the CEP units might need to be artificially enhanced during some periods,
by means of additions of soluble phosphate or nitrate. We also wanted to simulate N:P ratios
that might be encountered at other locations, where the influent may range from domestic
sewage discharge to agricultural drainage waters. Typically, algal treatment units are not
phosphorus limited, since algae have the capability to adjust to a wide range of N:P ratios present
in the water. Also, some internal recycling of phosphorus within the treatment units is common
after several months of operation, and if necessary, phosphorus can be recycled from nitrogen-
depleted algal sludge using anaerobic digestion and ammonia stripping.

We operated the CEP units under a variety of N:P ratios for a period of approximately 12 months. N:P ratios were determined weekly by centrifuging an aliquot of algal channel water and analyzing the total nitrogen and total phosphorus content of the settled algae and the supernatant. The weekly N:P ratios of the settled algae are plotted and compared to the N:P ratios of the incoming water supply in Figure 15.

Figure 15. N:P ratios found in algal tissue and in centrifuged supernatant as a function of the N:P ratio in the Whitewater River water supplied to the CEP. N:P ratios above 13:1 or below 8:1 were simulated by chemical additions of N or P as necessary. The N:P ratio of the algae was constant over a wide range of N:P in the supply water.
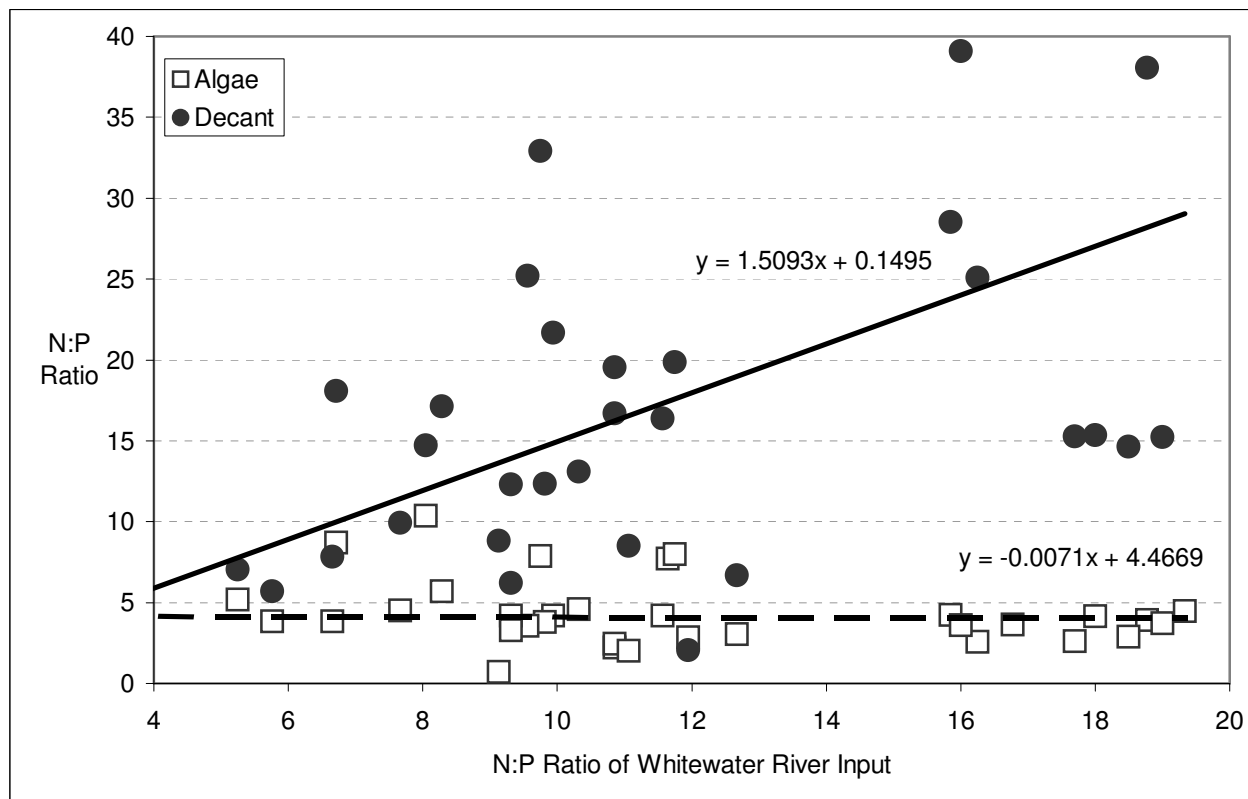
As seen in Figure 15, the N:P ratio of the algal biomass appeared to be largely independent of the incoming water supply for N:P ratios equal to or greater than 5:1. The algal N:P ratio was stable at about 5:1 while the incoming water ranged from 8:1 to 13:1. We also conducted tests at higher N:P ratios by artificially adding small amounts of nitrogen (urea) to the incoming water supply, and at lower N:P ratios by adding ammonium phosphate. Even under these more extreme conditions, the algal biomass was synthesized at an internal N:P ratio of approximately 5:1.

Based on these observations, we can make some projections regarding the applicability of the CEP process on the other tributaries of the Salton Sea. Table 3 summarizes data for the N, P, and N:P ratios for the New, Alamo, and Whitewater Rivers. It appears that the three major sources of water flowing into the Sea all have N:P ratios in excess of 10:1, whereas the data collected during this study indicate that in large-scale applications of CEP, for any given removal

rate of phosphorus, nitrogen will be removed at a rate approximately 5 times greater. Therefore, given the available N:P ratio data indicating that there is 10.4 - 18.9 times more nitrogen available, there will always be an excess of nitrogen present and phosphorus will be the limiting nutrient in CEP operation. This indicates that additions of nitrogen should not be required in large-scale operation. From a phosphorus removal standpoint, the observed 5:1 ratio of nitrogen to phosphorus suggests that there is sufficient nitrogen in the incoming water to facilitate maximum removal of the phosphorus.

Table 3. Measurements of nitrogen, phosphorus, and N:P ratio in the three major tributaries to the Salton Sea.

	Total N	Total P	N:P ratio
New River (Holdren 1999)	11.4	1.1	10.4 : 1
Alamo River (Holdren 1999)	10.5	0.7	15.0 : 1
Whitewater River (Holdren 1999)	17.0	0.9	18.9 : 1
Whitewater River (this study)	16.8	1.5	11.2 : 1

The data from Holden (1999) suggest that the N:P ratios of the New and Alamo rivers may be more favorable to total nutrient removal (including nitrogen), due to the lower levels of total N in these tributaries. The Whitewater River phosphorus data obtained during the current study appears to differ somewhat from the earlier information obtained by Holdren (1999).

B) Flow Rates and Hydraulic Retention Times

As applied to the CEP process, hydraulic retention time is the average length of time that influent water remains in the Algal Treatment Zone before passing on to the next treatment component. It is a function of flow rate and system volume. It is not affected by the rate at which the water is recycled within the Algal Treatment Zone by the primary paddlewheel. Based on preliminary estimates of nutrient concentrations in the three rivers and estimates of algal productivity that should be achievable in the CEP process, we have developed a target flow rate of 100 gpm per acre. For the 0.7 acre pilot scale CEP units under evaluation, filled to an average depth of 21 inches, the hydraulic retention time at 100 gpm/acre would be about 4.0 days.

Incoming Whitewater River water flow rates were slowly increased over the duration of the project so that we achieved the target flow rates. As indicated in Table 4, flow rates were increased from 57 gpm/acre to 85 gpm/acre and finally to 107 gpm/acre, while observing the effect on algal productivity and algal biomass.

Table 4. Effects of increasing flow on algal productivity in pilot-scale CEP units.

| Influent Flow Rate per Surface Acre	57 gpm	85 gpm	107 gpm
Average Productivity - g C /m^2 -day	*	*	*
Average Algal Biomass - mg VSS/L	*	*	*
Hydraulic Retention Time - days	7.0	4.7	3.7

(* - Data analysis in progress).

C) Flocculation Requirements

In order for the CEP units to achieve 80-90% removal of phosphorus, the discharge water from the CEP will need to be reduced to concentrations as low as 0.07 - 0.3 mg/L of total P. During the colder seasons there will be less algal productivity, making this level of conversion of nutrients to algal biomass more difficult to achieve. In addition, although the biological filter feeders used to harvest algae in the Secondary Fish Zone appear to be very efficient, there may be periods when a small amount of algal biomass that will not be removed by the Algal Sedimentation Belt. For these reasons, some form of additional chemical flocculation will most likely be required after the Secondary Fish Zone of the CEP process.

To determine the type and concentration of flocculants that would be useful during a winter period when there were no filter-feeding fish stocked in the CEP units, we conducted an evaluation of two commonly used coagulation-flocculation aids: alum and ferric chloride. Two sets of studies were conducted using water removed from the CEP Algal Treatment Zone. Preliminary experiments were completed in the laboratory with the aid of a jartest apparatus. After the laboratory-scale studies, additional trials were conducted in the 0.7 acre CEP units.

The laboratory flocculation studies involved measurements of soluble ortho phosphorus in samples taken from the Whitewater River, the CEP Algal Zone, and the Polishing Zone discharge. Soluble phosphorus was measured after the addition of settling agents to a one liter sample that was mixed continuously in a motorized jartesting apparatus. The results (Figures 16 and 17) were fairly linear and suggested that 20 mg of alum ($Al(SO_4)_3$ $18H_2O$) per liter was required to lower ortho phosphorus levels in the polishing and algal CEP water to less than 0.20 mg P per liter. The alum needed to lower the phosphorus concentration in the Whitewater River to less than 0.20 mg P per liter was 40 mg alum per liter. Ferric chloride requirements also appeared linear with dose. About 0.015 mg $FeCl_3$ per liter (3.5 molar) was required to lower the ortho phosphorus concentrations in the algal channel and the Polishing Zone water to less than 0.20 mg P per liter. The ferric chloride concentration required to lower the Whitewater River water to below 0.20 mg P per liter was about 0.030 mg $FeCl_3$ per liter. These data were utilized to determine the in-situ flocculant application rates to be evaluated in the 0.7 acre CEP.

Figures 16 and 17. Results of jartesting of alum (left) and ferric chloride (right) as flocculants to assist in settling algae populations present in CEP systems.
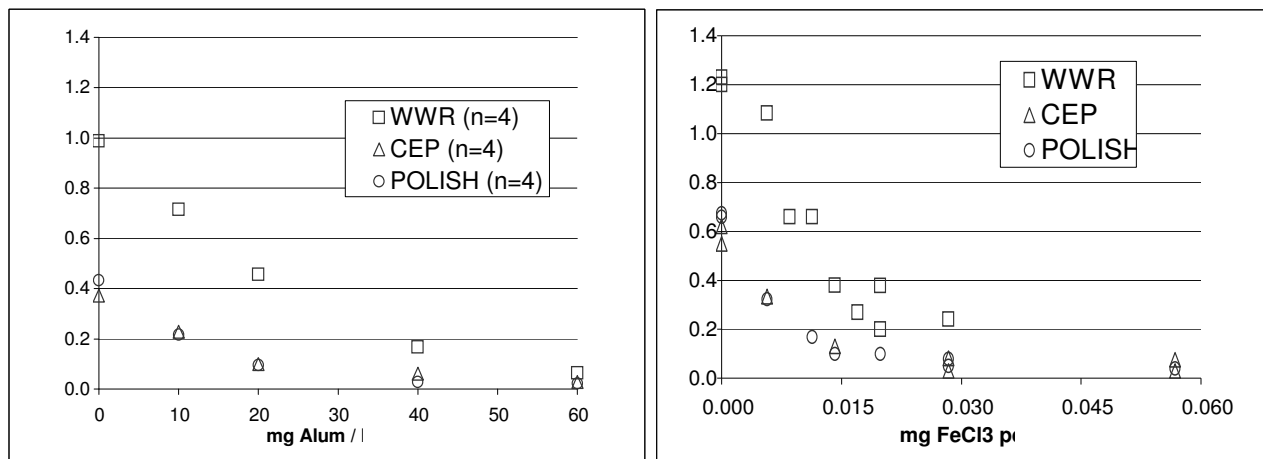
In situ flocculation experiments were conducted using a small flocculator mixing device that was installed directly in the concrete channel of one CEP unit. The data (Figure 18) indicated that for 90% removal of total phosphorus, approximately 0.17 ml of $FeCl_3$ would be required per liter of water. This agrees fairly well with the preliminary jartest estimates. In order to remove 90% of the soluble phosphorus fraction (ortho-P), the chemical requirement was reduced to 0.10 ml of $FeCl_3$ per liter of treatment water. Again, these preliminary tests were conducted on water diverted from the Algal Treatment Zone that had not received any polishing, as might occur in winter. The amount of flocculant required when treating the treated discharge from the Primary and Secondary Fish Zones of the CEP systems should be considerably less than these values. More detailed studies of flocculation requirements are planned for the winter of 2003 in cooperation with Dr. Chris Amrhein of UC Riverside.

Figure 18. Results of studies conducted in the CEP system on the use of ferric chloride as a flocculation agent to accelerate the settling of algae during winter.
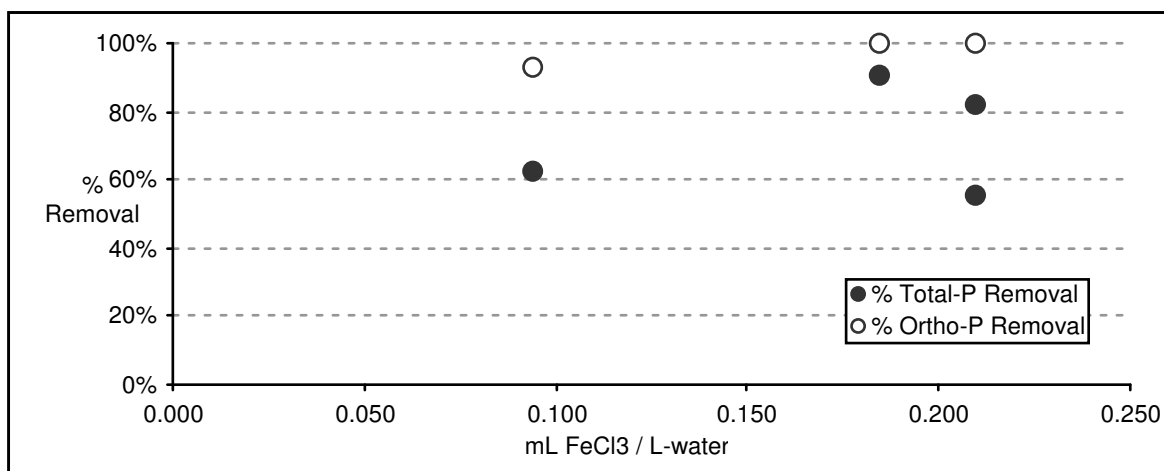
D) Internal Cycling of Phosphorus

Typically, the total phosphorus concentration in the Whitewater River input to the CEP system ranged between 1.5 and 1.75 mg/L, with soluble phosphorus levels in the CEP discharge ranging from 0.25 to 0.75 mg/L. This resulted in a net apparent algal incorporation of 50% to 85%. However, the total phosphorus concentration measured in the CEP Algal Zone could vary between 0.75 and 1.25 mg/L, which suggested an unaccounted-for phosphorus content of 20 to 50% of the input concentration. These differences indicate that particulate phosphorus present in the input water and a portion of the algal biomass growing in the CEP may be settling to the bottom. In addition, a fraction of the phosphorus input from the Whitewater River may be precipitating upon arrival in the CEP Algal Zone.

The observed standing algal biomass at any given time (in mg algal-C / L) can only account for about 50% of the observed productivity or carbon fixation. This suggests that a significant amount of the algal biomass could be settling in the CEP unit. Additionally, the observed nitrogen removal rates suggest the possibility of some nitrogen recycling. The nitrogen content

is likely lost from the settled algal biomass returning to the CEP water column as re-mineralized ammonia nitrogen, (increasing the nitrogen content of the discharge water). However, the sediment phosphorus content is apparently not being released, and consequently the CEP sediment is behaving as a long term trap for phosphorus, a secondary phosphorus removal technique that will be examined in more detail in subsequent studies.

Phosphorus precipitation could also be occurring. The difference between total phosphorus and ortho (soluble) phosphorus in the Whitewater River ranges between 0.2 to 0.4 mg/L. Some of the total phosphorus may be bound in particulate matter (sand particles) that settles easily in the CEP unit. In addition, the combination of high calcium in the Whitewater River and the potentially high pH present in the algal channel water column could promote phosphorus precipitation.

Sediment cores from inside the CEP unit and from around the earthen banks were collected and sent to an outside laboratory (Babcock and Sons, Riverside, CA) for measurement of total phosphorus. The results of these analyses suggested that elevated phosphorus concentrations did not appear to be present in the sediment of the CEP units. Whether a measurable portion of the phosphorus settles over time due to algal sedimentation or precipitation is an interesting concept for future evaluation, but this pathway may be of limited importance in terms of recycling, since it appears that once the phosphorus reaches the sediment, it is bound and does not easily re-mineralize. The nitrogen however, does appear to recycle to some extent, resulting in higher N:P ratios.
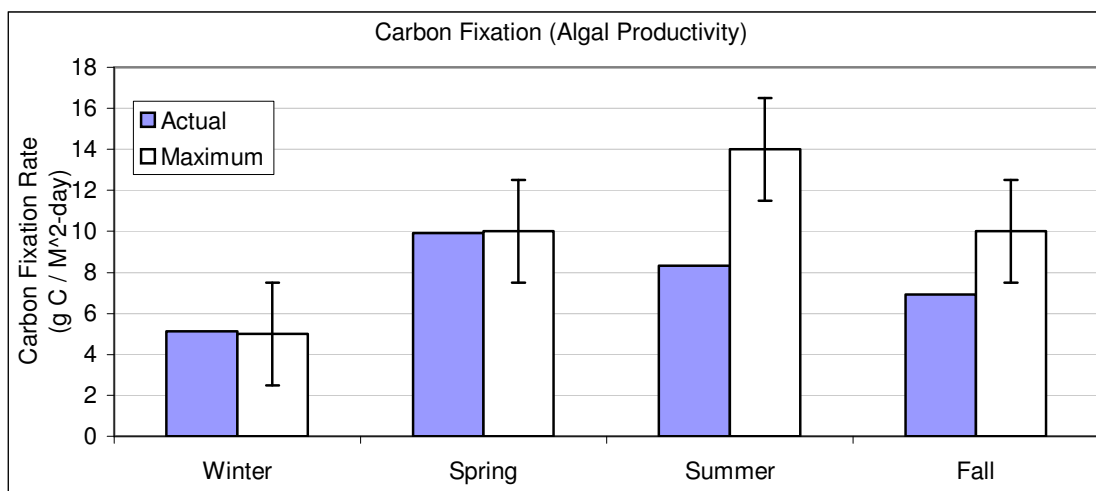
Task 4) Determine algal growth capacity, dominant algal species composition, and monitor uptake of selenium into algal and fish tissues.

A) Algal Growth Capacity and Productivity

The ability of the CEP system to remove nutrients such as phosphorus and nitrogen from surface waters is controlled by the rate at which photosynthetic algae are able to assimilate and convert these nutrients into biomass. In order to optimize the performance of the pilot scale CEP units, it was necessary to quantify variations in assimilation capacity and growth rate of the algae as impacted by CEP operating conditions and seasonal changes in temperature and sunlight. Light/dark bottle measurements were used to quantify algae growth rates and nutrient uptake in the CEP units by measuring oxygen productivity directly and then converting oxygen production into N or P uptake and biomass production through the use of field-determined stoichiometric relationships. Algal growth capacity was described in terms of the carbon fixation rate, which is the grams of carbon fixed (or assimilated into algal biomass) per square meter of pond surface area exposed to solar radiation per day. Photosynthesis and respiration were obtained by measuring in situ changes in oxygen concentrations occurring within 24 hr periods in a clear glass bottle (light bottle) and a darkened glass bottle (dark bottle). A net oxygen production is observed in the light bottle as a result of photosynthesis. In the dark bottle, oxygen is consumed due to respiration, carbon dioxide is released, and as a result a decline in oxygen concentration is observed. The net oxygen production obtained from these field measurements (photosynthesis minus respiration) is then used to calculate the overall net average carbon fixation rate. For the CEP system, a 1:1 molar ratio of carbon to oxygen can be used to convert oxygen production rate into the carbon fixation rate. The average carbon fixation rates measured in the CEP units are presented in Figure 19, together with a comparison of the typically observed maximum carbon fixation rates for outdoor large-scale algal mass culture systems.

The data in Figure 19 show the observed overall net production as impacted by seasonal climatic variations and algal density variations. Typical summertime sustainable fixation rates for outdoor mass culture systems can average 14 g C/m^2-day and reach a sustained maximum rate as high as 16 g C/m^2-day. As the season shifts to winter, water temperatures drop and solar radiation decreases, resulting in reduced carbon fixation rates. Sustainable winter fixation rates may average 5 g C/m^2-day and reach a maximum of 7 g C/m^2-day for the water temperatures measured at Mecca. Actual observed fixation rates for the winter of 2002-03 averaged 5.1 g C/m^2-day. Spring and fall fixation rates would be expected to average 10 g C $/m^2$-day, with a maximum sustainable rate of 12.5 g C/m^2-day. Actual observed fixation rates for spring and fall 2003 averaged 9.9 and 7.0 g C/m^2-day, respectively. Carbon fixation rates are typically expected to peak during the summer and average 14 g C $/m^2$-day; however, as a result of range-finding studies being conducted on high densities of filter-feeding fish in the CEP units during this time, the average fixation rates during the summer of 2003 were reduced to approximately 8 g C $/m^2$-day. This occurred while algal densities were reduced to levels below optimum until the end of summer, when the filter-feeding studies were completed.

Figure 19. Actual CEP carbon fixation rates for 2003 compared with typically observed productivities in large-scale mass culture operations, indicated by the range bars. In this study, fixation rates as high as 10 g C/m^2-day were observed during the first half of the year. Lower average values were observed during late summer as a result of intentional overharvest of the algal population by high densities of filter-feeding fish introduced during fish uptake trials (see text).

Carbon Fixation (Algal Productivity)

Carbon Fixation Rate (g C / M^2-day)

Legend:
- Actual
- Maximum

Categories: Winter, Spring, Summer, Fall

In non-mixed algal ponds, the carbon fixation can be cancelled out by algal respiration and the re-release of carbon back into the water column. For non-mixed ponds, a net carbon fixation rate of 1 to 3 g C/m^2-day is typically observed, even during the summer months. For mixed high-rate algal pond systems such as used in the CEP, the increased rapid algal growth results in a tremendous amount of oxygen production (leading to high carbon fixation and nutrient uptake). An essential component of the CEP design is to maintain low algal cell age so that the algae population is rapidly expanding. In an unmixed lake or pond, the algal cell age may be as old as fourteen days, while high-rate controlled systems are designed to maintain algal cell age between two and four days. The cell age can be manipulated by managing the hydraulic retention time or the cell retention time. The hydraulic retention time can be controlled by the adjusting the incoming water flow rates, while cell retention times are controlled using populations of filter-feeding fish. The combination of optimal flow rates and an optimal density of filter feeders is used to maintain a young and rapidly growing algal culture in the CEP system.

B) Dominant Algal Genera

As shown in Table 5 and Photograph 31, we were able to identify a variety of algae genera that grew within the CEP Algal Treatment Zones during the experimental trials. Of these, four genera appeared to dominate or were very abundant over the entire year: *Scenedesmus*, centric diatoms, pennate diatoms, and *Planktosphaeria*.

Table 5. Common genera of algae observed in the CEP units at Mecca.

Chlorophyta (Green Algae)	Chlorophyta (Green Algae)	Cyanophyta (Blue-green Algae)	Bacillariophyceae (Diatoms)	Euglenophyta (Phytoflagellates)
Ankistrodesmus Coelastrum Chlorella Micratinium Tetraedron	Pediastrum Scenedesmus Planktosphaeria Sphaerocystis Monoraphidium	Merismopedia Microcystis	Centric diatom Pennate diatom	Euglena

During the fall and winter months, a population of diatoms was observed to dominate the system, which reverted to primarily green algae genera as water temperatures increased. During this temperature and light-dependent secession of algal species, the observed nutrient assimilation capacity of the CEP system was not affected. Table 6 summarizes the changes in the dominant and abundant genera that were observed monthly during 2003.

Table 6. Changes in the dominant algal populations observed in the CEP systems during 2003.

Month	Dominant Genera	Abundant Genera
Jan	Planktosphaeria, Centric diatom	
Feb	centric diatom, Planktosphaeria	
March	Centric diatom	Planktosphaeria, Pennate diatom
April	Pennate diatom	Scenedesmus
May	Scenedesmus	Centric diatom
June	Scenedesmus	
July	Scenedesmus, Centric diatom	
August	Planktosphaeria	Scenedesmus, Centric diatom
September	Scenedesmus, Centric diatom	
October	Scenedesmus	Pediastrum

C) Uptake of Selenium into Algal and Fish Tissue

In any water treatment technology in which nutrients are concentrated for ultimate removal from the system, there is a potential for bioaccumulation of toxic compounds that could impact the usefulness of products obtained from the process. There was some concern that selenium, which is present in low levels in the Salton Sea tributaries, could become concentrated in the algae and/or the filter feeding fish and algae sludge. Our expectation is that algae may remove a minor amount of selenium currently flowing into the Sea, but not to such a degree that the algal byproducts or the filter-feeding fish produced become unusable. To confirm the degree of concentration of selenium that occurs in the CEP process, samples of the filter-feeding fish and the algal concentrate were taken periodically and frozen. At the conclusion of the research, these samples were analyzed by Michelson Laboratories in Los Angeles for total selenium content. In all samples (pre- and post-CEP treatment), the levels of selenium present in the fish tissue were below the level of detection (<50 ppb).

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Task 5. Evaluate the external carbon requirements of the system and filter feeder growth capacity.

In addition to phosphorus and nitrogen, carbon is required to maximize algal growth. In the Algal Treatment Zone of CEP, it is expected that carbon will be limiting for the process and will require supplementation. In most aquaculture CEP applications, fish respiration can provide the carbon dioxide needed for algae biosynthesis. However, the CEP configuration being evaluated for the Salton Sea requires (for maximum algal harvest and removal) that the Fish Zones be located downstream from the Algal Treatment Zone, thereby making recycling of respiratory carbon difficult. During this study, we determined the amount of supplemental carbon necessary in this application of CEP. Providing carbon in the form of gaseous carbon dioxide is an efficient way to get carbon into solution so that it is available for algal growth. In full-scale CEP units, the carbon needs could be met through recycling of carbon dioxide that would result from the combustion of methane (produced through the digestion of algal sludge) in electric generators.   Thus, the costs we incurred in supplementing carbon in these pilot-scale studies are not representative of the full-scale CEP implementation.
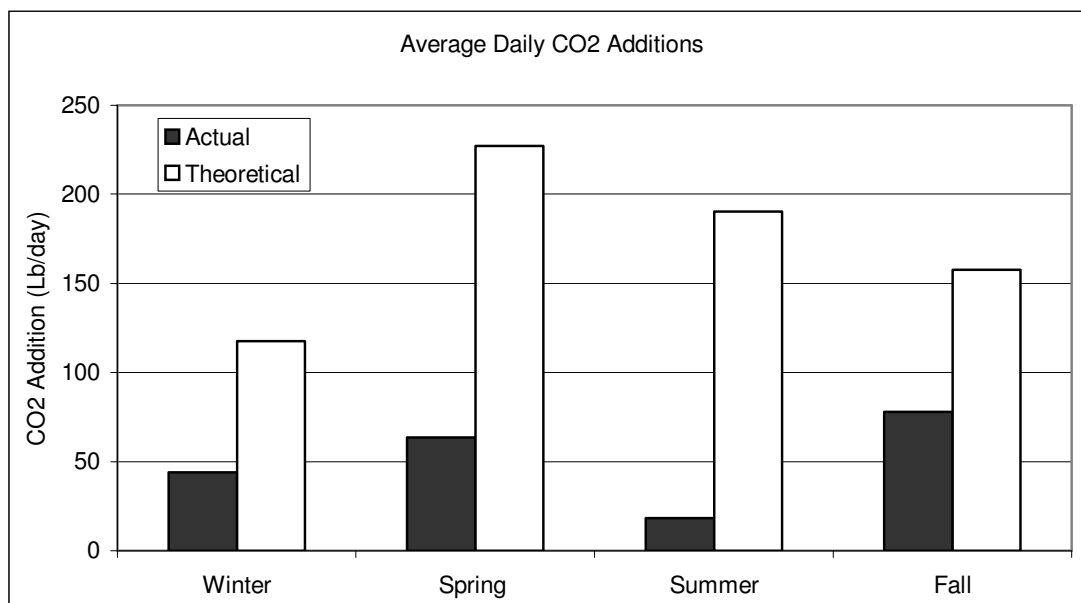
A) Carbon Dioxide Requirements

Theoretically, the supplemental carbon requirement will be equal to the carbon uptake or carbon fixation rate of the algae. For a 0.7 acre high-rate algal pond fixing carbon at an average rate of 12 g C /m^2-day, the required CO_2 could be as much as 275 lb per day. However, the actual carbon fixation rate is dependent upon temperature and solar radiation, and therefore the observed fixation rates vary seasonally. Also, for waters high in alkalinity, algae can obtain a portion of the required carbon from the incoming water. Figure 20 shows the actual daily carbon dioxide additions needed by the CEP units, compared to the theoretical daily requirement that would be needed to meet the fixation rates that were actually obtained in the units. As can be seen in the figure, only a portion of the carbon requirement of the algae needed to be supplied via supplementation.

The reduction in the actual CO_2 additions needed during the summer of 2003 is attributable to a variety of factors. For a portion of this time, algal productivity was low as a result of studies that were being conducted on the effects of overgrazing by high densities of filter-feeders. Due to the low algal biomass, the pH of the unit ranged between 8.5 and 9.0, indicating that the algal biomass may have been slightly carbon-limited.

Injection is sometimes complex, because the CO_2 additions should take place during the peak period of photosynthesis (about six hours at mid-day). It does little good to add CO_2 during the evening and night hours (the respiration stage), since the algae cannot utilize it at that time. Some CO_2 will be stored in the water column, but as the pH decreases due to CO_2 addition, the loss due to diffusion increases. To insure carbon limitation does not occur in the algal ponds, the pH should be kept below 8.0.

Figure 20. Average actual carbon dioxide additions supplied to the algal population in CEP systems at Mecca, compared to the total theoretical carbon dioxide requirement calculated from the actual carbon fixation rates measured in these systems.

B) Carbon Dioxide Production from Digestion Studies

During these studies, approximately 20 to 25% of the algal carbon requirement was supplied by external carbon dioxide injection into the CEP unit. The other 75 to 80% of the algal carbon supply came from the alkalinity of the incoming Whitewater River water. During anaerobic digestion, our studies showed that 50 to 60 % of the sludge carbon loaded into the digester is recoverable and would be available as CO_2 for re-carbonation of the growing algal culture. In the digester trials conducted at Clemson, it was discovered that improved gasification of algal biomass could be achieved by combining the algae sludge with a source of waste cellulose (agriculture waste, waste paper, or shredded municipal solid waste).

Using the combined algal/paper substrate concept, an anaerobic digester could be expected to yield all of the required CO_2 for recarbonation of the CEP algal culture if 30-40% of the algal biomass produced in the CEP system were run through the digestion process. In a commercial implementation of CEP at the Salton Sea, it is likely that sufficient algal biomass would be available to the digester to supply 100% of the carbon needs of the growing culture, completely eliminating external CO_2 requirements.

Task 6. Evaluate the use of automatic algal sedimentation belts for algal harvest.

Pond systems for mass culture of algae or use of algae to improve water quality both have a need for dependable and cost-effective methods of concentrating and removing or harvesting the unicellular algae that are produced. Harvest of billions of very small algal cells with a specific gravity almost identical to water is a difficult task. A variety of methods have been proposed, including filtration, centrifugation, and flocculation. Most previous methods are only applicable in situations where the product is valuable enough to justify these expensive forms of removal. In contrast to these methods, scientists at Clemson University have been developing a settling belt concept, in which the algae are encouraged to settle onto a mesh fabric belt located at the bottom of a tank or channel. A prototype algal harvest belt system was designed, installed, and operated at Clemson University's research facility (Photographs 26-28). Periodically, the belt is advanced by a gearmotor and the settled algal sludge is brought gently out of the water, where it is collected in a storage reservoir. The belt harvester advances slowly, at a speed of about 8 inches/min, and cleats installed transversely on the belt keep the sludge in place as it is lifted out of the water at a critical angle of 11 degrees. The belt harvester has been shown to be capable of delivering a concentrated algal paste that consists of 10-15% solids, both with and without the use of metal salts to enhance algal flocculation and settling.

During this project, studies were conducted at Clemson and at the Mecca facility of Kent SeaTech to improve the design and efficiency of the belt concept and adapt it for use as a component in the CEP treatment process for the Salton Sea's tributaries.

A) Design of Algal Sedimentation Belt

1) Improvements in Design

The Algal Sedimentation Belt that was installed in the existing CEP unit at Kent SeaTech was designed to fit in the concrete fish chambers that were already in place at the existing CEP units at Mecca. Given the 0.7 acre Algal Treatment Zone of these CEP units, the surface area of the belt was larger than necessary, but was still very useful in determining all the operational data concerning the belt concept. During the construction, operation, and modification of this prototype belt over a 12 month period, we gained valuable knowledge regarding sizing requirements, optimal travel velocity, water velocity, water flow direction, potential problem areas, and daily operational protocols. This information will be of assistance in designing full-scale belt systems, which require knowledge of the optimal length and width requirements for the belt, the placement of the drive drums, the optimal pore size for the belt fabric, the belt support/tracking system, and the physical removal of algal paste from the belt. We also determined many of the recommended operational procedures, including the preferred direction of belt travel relative to the water flow, flow velocities, the optimal distance between the belt and the bottom of the fish cages, and the preferred fish biomass for each fish compartment.

Early in our design and testing of the belt concept, we realized that for optimal removal of settled algal sludge, the belt should span the entire length and width of all of the fish cages, so that the overall length and width of the belt will be determined by the chamber dimensions that are needed to meet the requirements of the fish populations. A very large amount of settled material

was deposited directly underneath the cages spanning the entire length of the belt. The volume of waste removed was observed to be as much as 40 to 50 kg of concentrated sludge (15-20% solids) per day per 0.7 acre CEP unit. During the first few months of operation, algal material was adhering to the belt and a portion was being returned to the water, causing a build-up of sludge around the point where the belt exited the Fish Zones. Also, build-up of a solid cake on the surface of the underwater free wheeling drum at the head of the belt system resulted in a gradual tightening of the belt, requiring periodic adjustments. If this problem became too pronounced, it was possible for some of the nylon cleats to be caught on the wheel frame mechanism at the point of incline. The cleats are used to prevent the algae from sloughing backwards while traveling up the incline surface and out of the water. To prevent algal buildup from accumulating on the belt and the drive rollers, a cross conveyor was constructed to actively sweep the belt clean before the belt returned to the water (see next section). In future designs, the underwater free wheeling roller drum probably should be located out of the water for more convenient servicing, or should be porous so that any build-up of compressed material will fall to the inside of the drum and can be periodically removed.

The sedimentation belt itself is constructed using a porous filter press material with the nylon cleats attached at nine inch increments. It rides a track made from angle iron with cross braces installed every 10 feet for support. In our tests, an incline angle of 11 degrees appeared to be sufficient to remove solids efficiently without significant slippage. At the point of incline, three wheels on a transverse spindle are used to hold the belt down on the track, since otherwise it would tend to bow upward. Due to the width of the current chamber the three wheels were required, but in future applications we believe that two wheels spaced one-third of the way in from each wall will be sufficient.

After consideration of several configurations, we believe that the best mode of operation will be for the direction of belt movement to be counter to the direction of water flow. This countercurrent design will help to prevent any algal biomass that might be resuspended as the belt rises out of the water column from entering the system's treated water drainpipe. A belt velocity of nine inches per minute (0.0125 ft/sec) did not stir up the settled algal paste, and we determined that the water velocity through the channel should not exceed 0.1 to 0.15 feet per second. In the countercurrent configuration, the total water velocity that is experienced by the settled algae would be the sum of the two opposing velocities, or about 0.11-0.16 ft/sec. Also, to prevent fish movements (in the cages above) from disturbing the settled algal biomass on the belt, we determined that the belt tracking system should be located approximately 6 inches below the bottom of the fish cages.

2) Addition of Cross Conveyor

At the beginning of the belt evaluations, we allowed the concentrated algal sludge to fall from the end of the belt to the storage reservoir by gravity, but soon noticed that a portion tended to adhere to the belt and return to the water. To enhance the removal of the algal paste from the belt surface, a cross conveyor was designed and constructed to actively sweep the settled algal paste off of the flat top surface of the belt before it contacted the drive roller (Photograph 16). The cross conveyor should be located on a flat (horizontal) section of belt because it is so efficient in gathering and sweeping the sludge that it creates a small mound of material that is

higher than the cleats and if there were an incline present, some of the collected material would slide down the belt back toward the water surface. The cross conveyor design has proved to be very efficient in reducing the amount of settled material that is returned back to the water.

B) Drag-chain Scraper Alternatives

Drag chain scrapers are sometimes used in conventional wastewater treatment plants to transport settled material and may have applications in CEP treatment. However, the algal waste is best removed via a dewatering belt in order to maintain a high percentage of solids. If the sludge were pumped out of the chamber as a slurry, the total volume of waste to be dealt with could be as much as 10-100 times greater than achieved using the belt. One potential use of drag chain scrapers might be to drag the bottom of the concrete fish zones to the end of the channel and then deposit the settled and scraped algae onto a sedimentation belt that would be slightly lower, and would then angle up and out of the water to be dewatered and concentrated (Figure 35). The advantage of the drag chain system would be in significant cost savings, since the filter press material needed for the belt is quite expensive. If a drag chain could be used, the length of the belt needed could be reduced to just an inclined ramp long enough to rise the height of the channel at an 11 degree angle. In ongoing studies, we will be evaluating this concept in order to determine if the drag-chain can transport settled algae along the bottom without resuspending it.

C) Algal Belt Studies at Clemson University

In order to develop operational data to assist in designing efficient algal sedimentation belts for use in full-scale CEP systems to be located at the Salton Sea, several studies of belt design and operation were conducted in three small (0.33 acre) CEP units located at the Clemson University aquaculture field station in South Carolina (Photograph 25).

An algal harvest belt measuring 50 ft by 3 ft was installed in each of the three separate Clemson CEP systems (Photograph 26). Two of the belts were operated with 114 kg of Nile tilapia (with an average weight of 250 g) confined in net cages that were located over the belts. One belt was operated using unaided settling/sedimentation as the only algae removal technique. The two tilapia-belt removal systems removed an average of 23 to 30 kg per day of algal solids from the 1/3 acre units. Due to the fact that the belt design is able to gently remove the algae from the water column and dewater it, the algal biomass became quite concentrated and consisted of 10 to 15% total solids, of which 40-80% was volatile solids (Figure 21). At this time, the CEP units were loaded with nitrogen and phosphorus that could support a carbon fixation rate of 3.5 to 7.0 gm C/m^2 day, while the actual observed average carbon fixation rate was 6.7 gm C/m^2 day at algal Secchi Disk Visibility readings of 5 – 15 cm and ammonia concentrations of 0.5 to 2.5 mg/L (Figures 22 and 23). The observed algal removal rate by the small prototype belts corresponded to approximately 10% of the actual observed algal fixation rate at a filter-feeder stocking rate equal to 842 kg wet weight per hectare (750 lb/acre). These data suggest that to achieve 90% removal of the algal solids present would require approximately 5,000 to 7,500 lb per acre of large tilapia (250 gm), as predicted by controlled experiments in which we measured the algal uptake rates of the fish. However, data from Clemson and Kent Sea Tech studies suggest that the fish biomass requirement for 90% algal removal may be reduced by 2/3 if smaller tilapia (60 g or less) were used instead of the larger fish available for this field study. In

South Carolina climatic conditions, algal paste of 15% solids was achieved, while under California climatic conditions, a paste as dry as 20% solids content was produced. The sedimentation belt that was operated without caged tilapia suspended above it was largely ineffective at removing algal solids, achieving less than 5% of the algal removal rate achieved by the tilapia – coupled belts.

Figure 21. Percent of volatile solids present in algal sludge total solids harvested by Algal Sedimentation Belt.
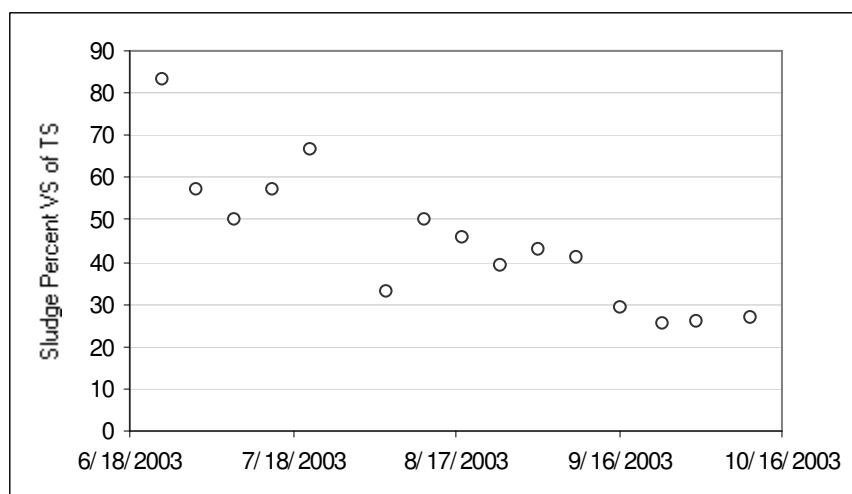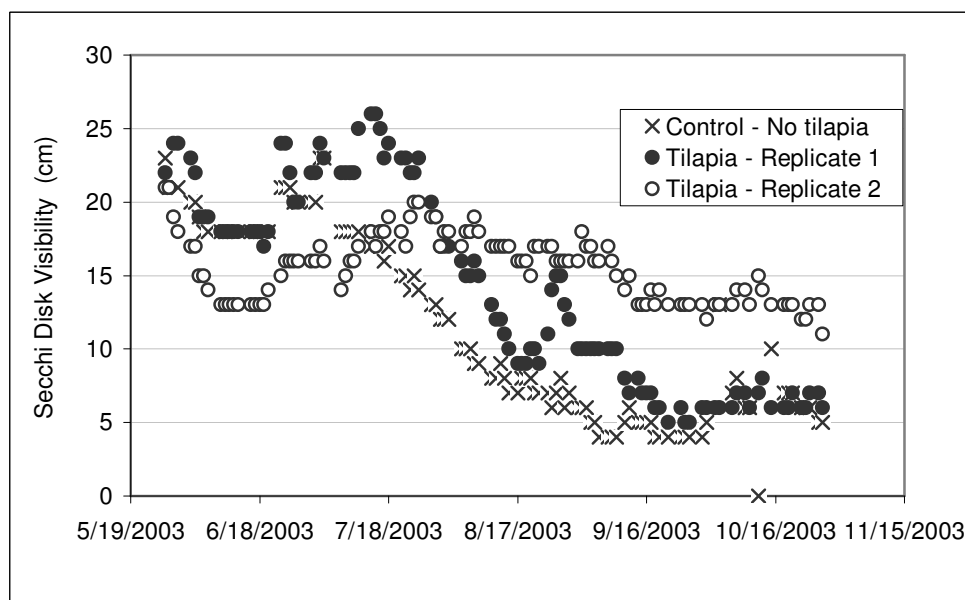

Figure 22. Secchi Disk Visibility readings in three CEP units equipped with Algal Sedimentation Belts.

The impact of tilapia harvest on the water column SDV can be seen in Figure 22. The CEP unit without tilapia over the belt stabilized at a lower SDV value (higher VSS) than the two units with

tilapia over the belt. This unit stabilized at a SDV of about 4 to 6 cm, while the units with fish stabilized at 6 to 12 cm. The ability to harvest algae is an important factor in the CEP process. By removing the algae, a low cell age is maintained, resulting in rapid growth of the algae and maximizing the nutrient uptake rate. By not harvesting algae, the algal standing crop will increase, as will the algal cell age. The overall effect is a decreased rate of nutrient removal because the individual algal growth rate decreases.

Figure 23. Ammonia concentrations measured in three CEP units equipped with Algal Sedimentation Belts.
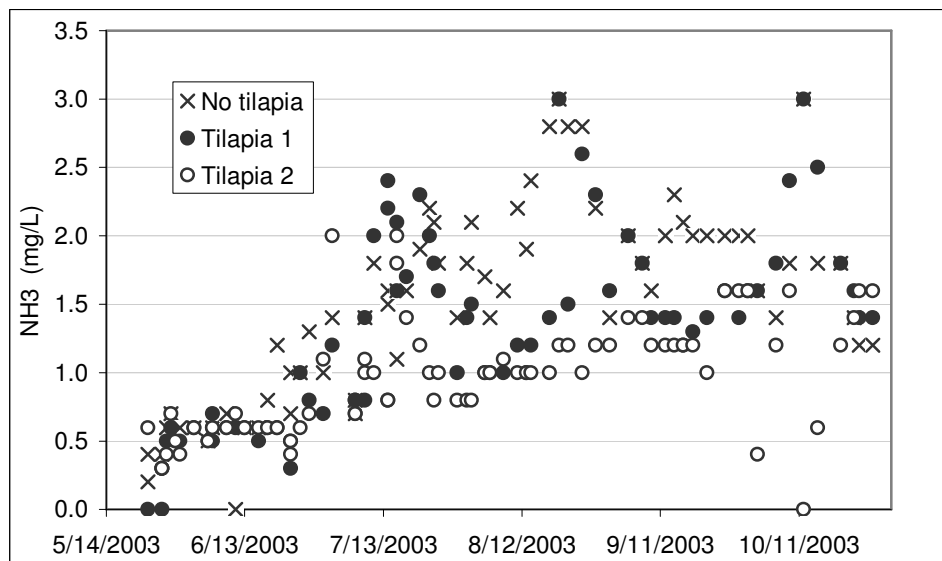
The frequency of algal harvest appeared to affect the dewatering ability of the belt. Initially, the belt was operated twice weekly, resulting in sludge with a solids concentration of 15%. As the frequency of harvest increased, the percent solids decreased. This probably was a result of compression settling that occurred while the sludge was on the belt between harvests. Algal harvest every two days resulted in 10% solids concentration, and daily harvest resulted in 6% solids. The impact of harvesting every day was a much lower solids concentration, resulting in a greater volume of algal for disposal or transport to a digester.

Task 7. Quantify conversion of algal biosolids into tilapia biomass and N and P concentrate for use as agriculture fertilizer and biofuels.

A) Conversion of Algal Biomass to Fish Biomass

1) Growth of Fish

Fish stocked in the CEP system averaged 16 grams and were sampled five times over the growing season. At the time of the last sample (October 23, 2003) the fish still averaged 16 grams. The average change in weight between the first and last sample ranged from –1.6 grams to +1.4 grams for the four different groups of fish studied. There was little growth documented

for the fish populations during these studies, due to several factors. First, the densities of the tilapia in the Primary and Secondary Fish Zones have been changed many times during these evaluations, since the primary objective has been to determine the optimal parameters for CEP operation to maximize the removal of phosphorus. As a result of these frequent changes in density and restocking, there has not been sufficient time in any single operating mode to measure a significant change in average fish weight. Also, we have learned a considerable amount about the methods for optimizing the algal feeding of this species of tilapia (*Oreochromis mossambicus*) and realize that in order to keep the fish population consuming algal cells efficiently, the densities encountered in the Secondary (Polishing) Fish Zone must be kept in careful balance. This induces the fish to graze upon the low density of algal cells available, which requires a considerable expenditure of energy, thereby limiting growth. We are developing a plan for rotating the cages of fish between the Primary and Secondary Fish Zones in order to achieve the desired high efficiency in algal consumption and yet still allow the population to grow. At this point, the rotation plan is under development and the results are not yet available.

2) Efficiency of Algal Grazing in Clemson Evaluation System

Filter feeding experiments were conducted at Clemson University to determine the rate at which tilapia are able to remove algae from water taken from the Clemson University CEP units. Six Continuous Stirred Tank Reactor tanks (CSTR) of 127 liters were stocked with juvenile Nile tilapia (69 g average weight), at a total biomass of 1.5 kg. A seventh CSTR without fish was used as a control. CEP water flow rates into each CSTR were 0.15, 0.5, 1.0, 1.5, 2.0 and 3.0 L/min during a 58 hr experimental period. Water was discharged through a standpipe and an airstone helped to maintain a mixed water column. Tilapia were held off the bottom by screening to avoid resuspension of feces. Water temperatures were recorded at 4 hr intervals from 0800 to 2000 during the experimental period. Dissolved oxygen, pH, and total ammonia nitrogen were measured at the beginning and ending of each trial.

Water quality (dissolved oxygen, pH, and ammonia nitrogen) parameters were statistically similar among experiments. The difference in particulate organic carbon concentration (POC) between the incoming and outgoing water in the control was not significant, indicating that phytoplankton suspensions were not affected by sedimentation or wall attachment. Nile tilapia filtration rates of green algae and cyanobacteria at each flow rate were linear from 17 to 32 °C. The intercepts and slopes of the linear regression were significantly different at 0.15, 0.5 and 1.0 L/min flows, while similar with flow rates 1.5 L/min and higher. Filtration rates achieved in this study were excellent (Photograph 35) The rates measured at the higher flow rates were similar and are represented by a single regression line in Figure 24.

Figure 24. Filtration rate vs. temperature in tilapia filtration trials at Clemson University.
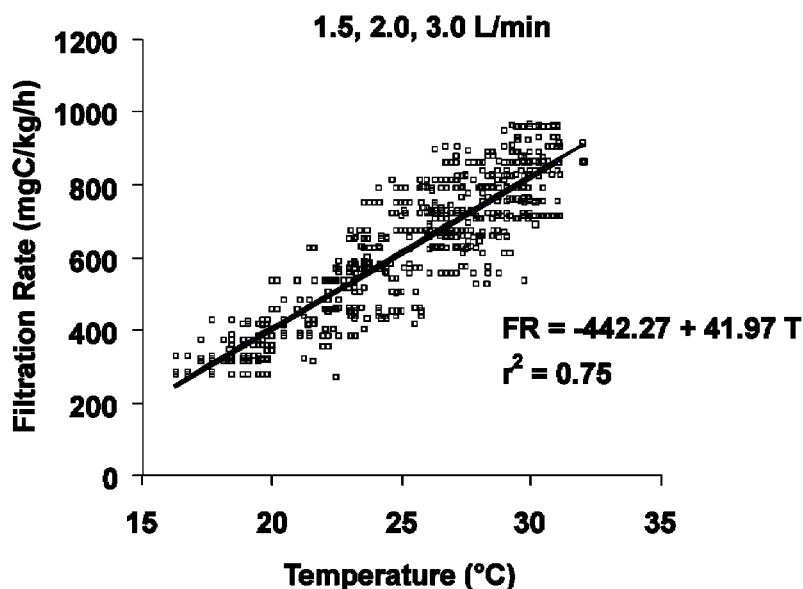
Similar to other tilapias, Nile tilapia filtration rate increased linearly with increasing water temperature. In studies by Mironova (1974), the food consumption rate of *O. mossambicus* increased linearly from 22 to 31 °C. The stomach contents of *S. galilaeus* were shown to increase linearly from 19.5 to 29.9 °C (Lauzanne 1978). The feeding rate of juvenile *T. rendalli* also increased from 18 to 34 °C, but as water temperatures exceeded 34 °C, feeding activity decreased (Caulton 1982). Bhikajee and Gobin (1997) observed a maximum feeding rate for red tilapia hybrids at 32 °C, which suggested that further temperature increases would depress feeding. Water temperatures (> 32.5 °C) were not observed in this study, but based on published reports for other tilapia species, Nile tilapia filtration rates would be expected to decrease at higher water temperatures.

Filtration rate can be described as a non-linear function of POC concentration using Ivlev's (1961) model. Nile tilapia filtration rates increased with increases in POC concentration and the rate of increase (lower half-saturation POC) was faster in a warm-water regime than in cool water. Filtration rates eventually leveled off with further increases in POC concentration in each temperature regime. The same filtration rate pattern with increased POC concentration and water temperature was observed with both green-algal and cyanobacterial water sources (Figures 25 and 26).

Ivlev's (1961) model indicates that both POC concentration and temperature affect tilapia filtration rate and thus both will affect removal of phytoplankton from the CEP. Determining the maximum filtration rates will allow us to predict the impact of POC values and temperature upon filter feeder algal harvest rates and resulting algal standing crop as situations vary in the CEP units.

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Figure 25. Filtration Rate vs. Particulate Organic Carbon for Green Algae.
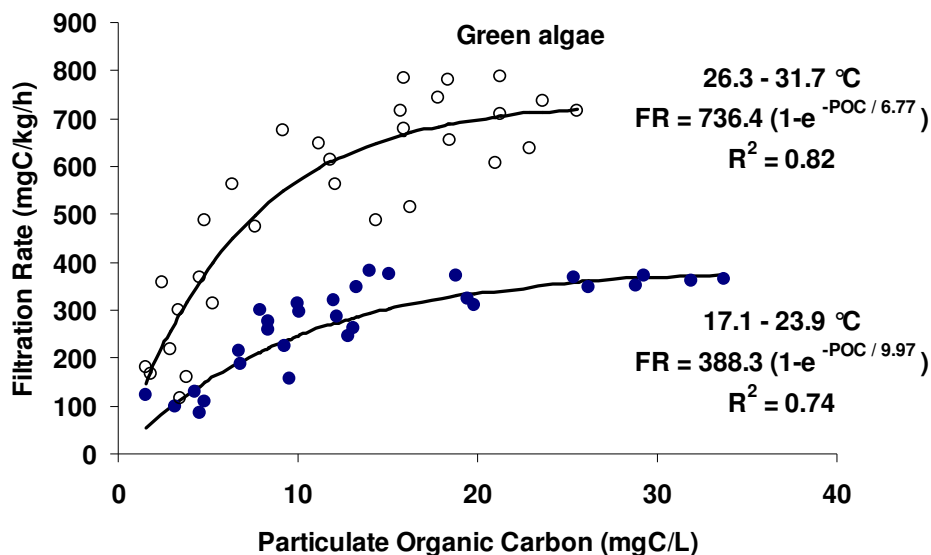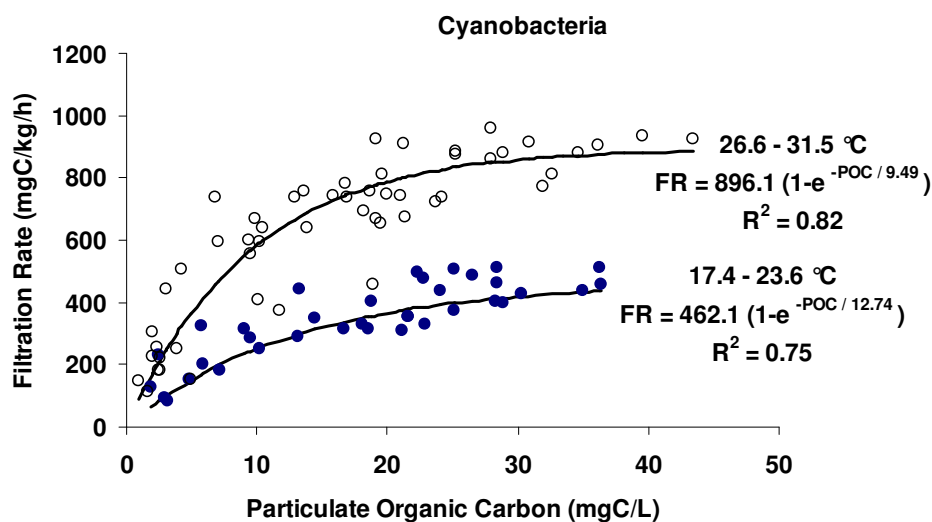
Green algae

$$FR = 736.4 \, (1-e^{-POC/6.77})$$

26.3 - 31.7 ℃

$R^2 = 0.82$

$$FR = 388.3 \, (1-e^{-POC/9.97})$$

17.1 - 23.9 ℃

$R^2 = 0.74$

Filtration Rate (mgC/kg/h)

Particulate Organic Carbon (mgC/L)

Figure 26. Filtration Rate vs. Particulate Organic Carbon for Cyanobacteria.

Cyanobacteria

$$FR = 896.1 \, (1-e^{-POC/9.49})$$

26.6 - 31.5 ℃

$R^2 = 0.82$

$$FR = 462.1 \, (1-e^{-POC/12.74})$$

17.4 - 23.6 ℃

$R^2 = 0.75$

Filtration Rate (mgC/kg/h)

Particulate Organic Carbon (mgC/L)

3) Efficiency of Algal Grazing in Kent SeaTech Evaluation System

Filter-feeding experiments were conducted on the local strain of tilapia (a hybrid of *Oreochromis mossambicus*) at the Kent SeaTech facility in Mecca. Three 1,100 liter poly tanks adjacent to the 0.7 acre CEP units were used for these studies. Each of the tanks was initially stocked with 54 lb of tilapia (29 g average weight). Water flow rates into the tanks ranged from 7 to 27 liters per minute. Five experiments were run between July 23 and August 2, 2003. Water quality

measurements were taken of inflow TSS and VSS, outflow TSS and VSS, and the water flow-through rate. The data on the changes in VSS caused by fish grazing are shown in Figure 27.

> Figure 27. Inflow and outflow VSS levels in five trials to determine the filtration rate of tilapia in removing algae from water taken from the CEP Algal Treatment Zone. The average percent removal of VSS for three replicates is shown above the VSS out bars.
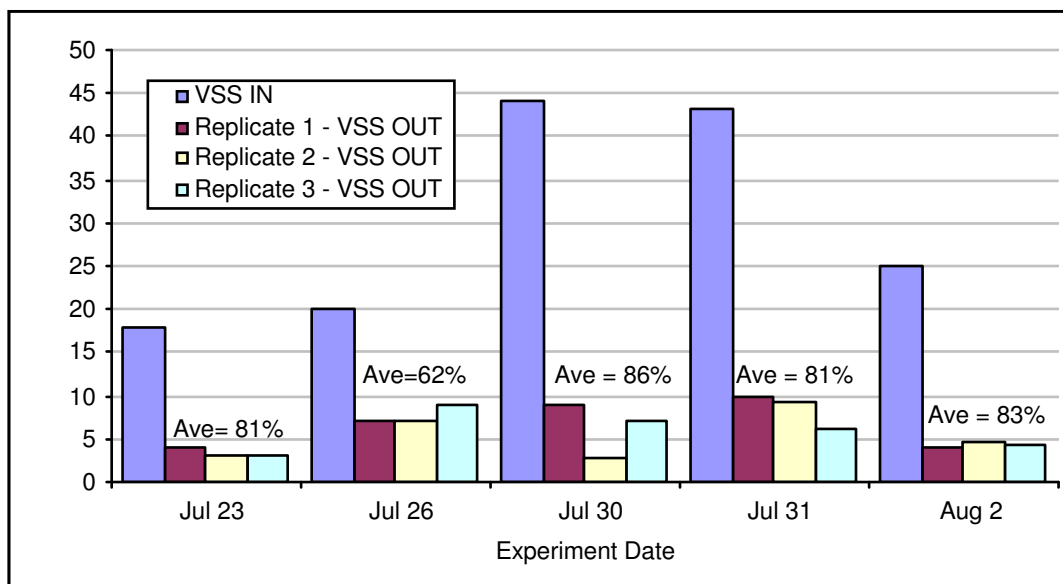

In these studies, the incoming VSS ranged from 18 to 44 mg/L, while the outgoing VSS after the tilapia consumed the algae was below 10 mg/L for all trials. For the flow rates and incoming VSS concentrations studied, the tilapia were able to remove as much as 86% of the incoming VSS. The outflow algal concentrations are similar to what would be expected in the Secondary Fish Zone of the CEP units. These "polishing" fish routinely remove 80% or more of the incoming algal VSS. More studies need to be conducted using higher flow rates (~40 L/min) to better quantify the VSS removal capabilities of tilapia held in the Primary Fish Zone.

From Figure 26, it can be seen that the maximum filtration rate for tilapia in warm water is approximately 800 mg C/kg-hr, which is equivalent to approximately 1,600 mg VSS/kg-hr. This is the maximum algal uptake rate as determined by the specific kinetic parameters of the fish. However, tilapia cannot achieve this maximal value if the mass flow rate of algae into the CEP unit is less than their filtration capacity. For the Secondary or Polishing fish in the CEP unit, this will always be the case. The maximum filtration capacity of these fish is described as "detention-time-dependent" because at the low flow rates present in the Secondary Fish Zone, any incoming concentration of VSS will be grazed down to very low levels (and therefore become limiting) due to the long detention time. Conversely, the Primary (or mass removal) fish will be exposed to the highest possible levels of algal VSS and therefore should continuously perform at 1,600 mg VSS/kg-hr, or at the filtration rate dictated by the kinetic parameters of the species. The filtration rate for these fish is dependent on the maximum uptake for these fish and is said to be "saturation-level-dependent". The feeding activity of these fish will make little difference in the overall algal VSS concentration observed in the unit, but the total mass of algae they will consume will be quite large. For any algal mass flow rate into the fish zones that is

between these two levels, (detention-time-dependent and saturation-level-dependent), the fish filtration rate will be dependent on the algal concentration.

The filtration rate, in mg C/kg/hr, was calculated for these experimental groups and is shown in Figure 28. The data was separated into two groups based on the incoming water flow rate. The low flow rate category received water flow of less than 10 liters per minute, while the medium flow rate category received 11 to 27 liters per minute of incoming flow from the CEP units.

Figure 28. Filtration rate of tilapia receiving water from the CEP Algal Treatment Zone at low and medium water flow rates. The particulate organic carbon concentrations were estimated from measurements of VSS.
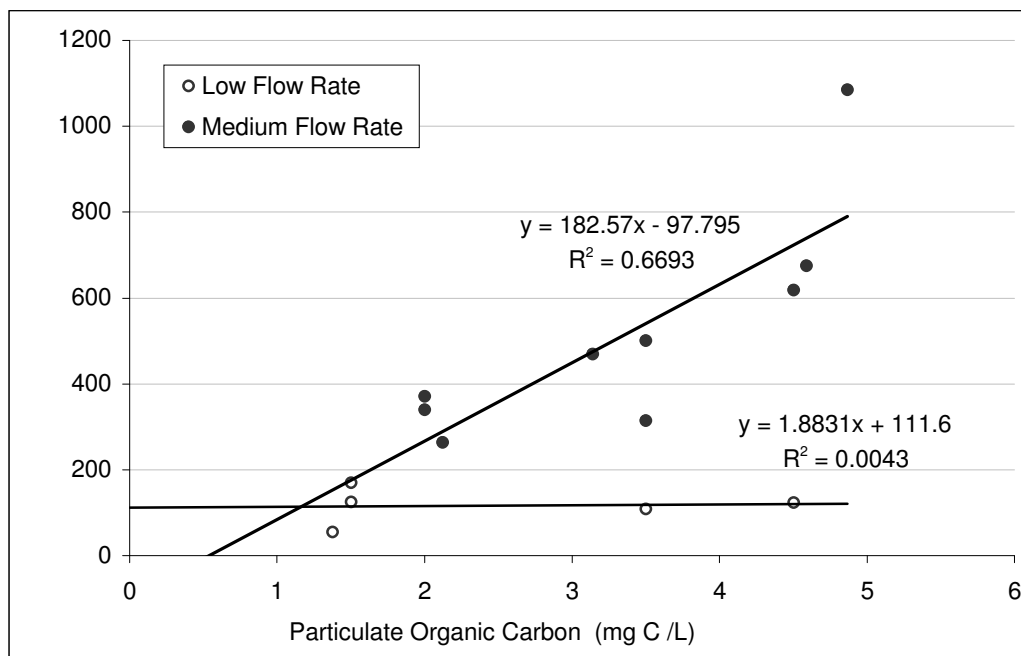

These data illustrate that filtration rates can differ depending on the incoming flow rate and how it affects the detention time. The low-flow, high-detention-time fish represent the Secondary or polishing fish in a CEP application. The filtration rate of these fish is limited by the inadequate mass of algae supplied by the low flow rates. In these trials, the fish filtration rates are constant at about 110 mg C/kg/hr, even though the tank algal concentration was tripled. The mass flow rate of algae into the system is the limiting factor in the low flow rate condition, and as stated above, these fish are detention-time-dependent.

In contrast, the filtration rate of the fish in the tanks receiving medium incoming water flow appears limited by the algal concentration. The fish filtration rate is a function of algal concentration, so that the higher the concentration, the higher the filtration rate. The discharge algal concentrations ranged from 1.5 to 5 mgC /L in these trials, while the filtration rates ranged from 200 to 1,100 mgC/kg/hr. These filtration rate studies were conducted at relatively low algae concentrations, and the results are similar to those collected at Clemson University. As reported in the following section, additional studies were performed at higher algae concentrations to better determine the maximum algal uptake rate.

4) Efficiency of Algal Grazing in CEP Fish Channel

We also conducted studies of the filtration rate of tilapia held in the fish zones of the 0.7 acre
CEP units, by measuring TSS and VSS into and out of each fish zone together with the
corresponding water flow rate. In these trials, the Primary Fish Zone held one cage of tilapia
with a total weight varying from 200 to 400 lb and the Secondary Fish Zone consisted of four
cages each containing 300 to 500 lb. Fish filtration rate was plotted as a function of the
discharge particulate organic carbon concentration (Figure 29).

Figure 29. Filtration rate of tilapia held in the CEP Fish Zones as a function
of the concentration of algae present. POC and FR in this study were
estimated from VSS measurements.

$$FR = 610 (1-e^{\wedge}-POC/5.2)$$
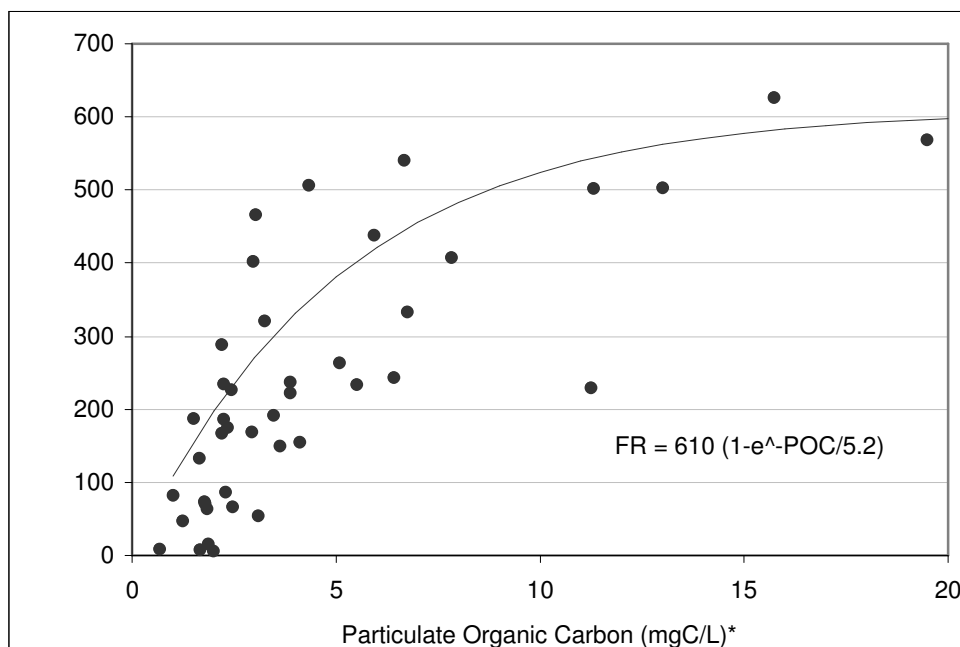
These data for the filtration rate of the local tilapia strain (*Oreochromis mossambicus*) held in the
CEP system appear similar to data obtained for Nile tilapia held in experimental systems at
Clemson University.

We also evaluated the changes in VSS concentration that occur as the water passes through the
Primary (Mass Removal) and Secondary (Polishing) Fish Zones in the 0.7 acre CEP units
(Figure 30). The low flow conditions encountered in the Secondary Fish Zone resulted in a high
detention time that allowed the tilapia to consume nearly all of the algae present, regardless of
concentration, while the tilapia in the Primary Fish Zone showed a consumption pattern that was
proportional to the incoming VSS concentration. In some of the trials, the mass removal fish
filtered the algae even better than anticipated, so that fish had to be removed from cages to avoid
significantly reducing the algal population in the Algal Treatment Zone. Both the Primary and
Secondary fish groups in the CEP systems appear to be performing well (Photographs 34 and
36). They are maintaining final algal concentrations at 3 to 8 mg VSS/L, even when the
incoming VSS is greater than 40 mg/L.

Figure 30. Change in VSS concentration as water passes through the Primary and Secondary Fish Zones of 0.7 acre CEP unit.
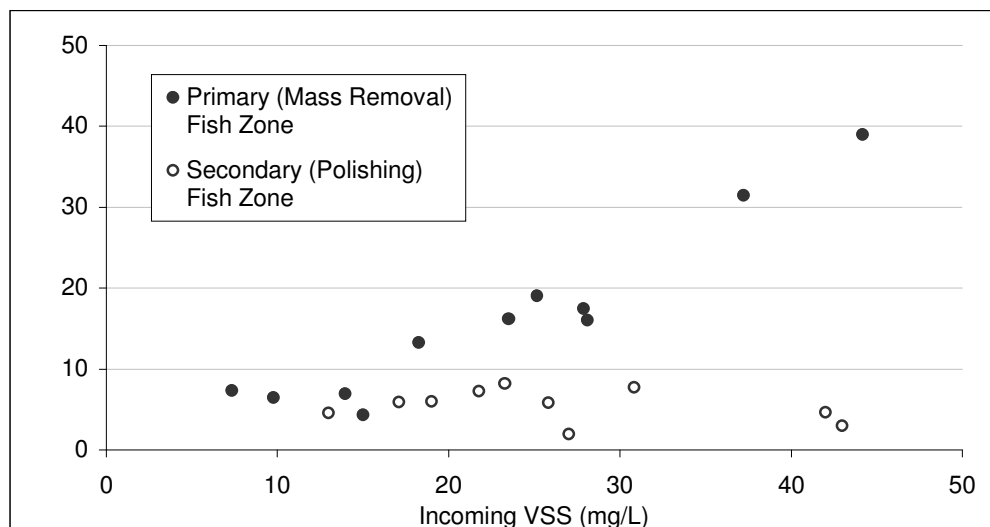

B) Alternative Filter-Feeders

The local strain of tilapia (Photograph 32) proved to be an excellent filter-feeding species for the CEP system during these studies (Photographs 34 and 36).   High filtration rates were achieved and we were able to reduce the density of fish and still obtain excellent removal of algae. However, tilapia are a tropical species and can present some disadvantages for year-round use in CEP systems. Although they can survive well during the summer even when water temperatures in shallow ponds exceed 37°C, when winter water temperatures drop below 14-18°C, tilapia suffer reduced growth and high mortalities. For this reason, we evaluated several alternative filter-feeding fish for use in the Secondary Fish Zone of the CEP system. We conducted a tank study of the filtration rate of the Sacramento blackfish, *Orthodon microlepidotus*, a native California cyprinid (Photographs 33 and 36, Figure 31). While primarily a zooplankton filter-feeding fish similar in nature to the Chinese bighead carp, its long intestine and fine gill raker structure are also adapted to feeding on algae. Sacramento blackfish can survive in water temperatures from near freezing to over 30°C. It has an added benefit of being able to survive and feed in water with dissolved oxygen concentrations below 1.0 mg/L. Our studies indicated that Sacramento blackfish may have applicability in CEP systems, although the results of the filtration trials were not consistent and more research will be necessary to understand how to best manage them in this application.

Our colleague Dr. Arne Eversole at Clemson University has studied the use of freshwater mussels and other bivalves in the CEP process and found that these organisms also can remove algal cells efficiently. In California, *Corbicula* freshwater clams are present that may be able to serve a similar function. We conducted preliminary trials with this species, but like the blackfish, additional studies will be needed to determine how to utilize them as filter feeders in the CEP process.

Figure 31. The filtration rate of Sacramento blackfish supplied with algae from the Algal Treatment Zone of the CEP system was inconsistent in these studies.
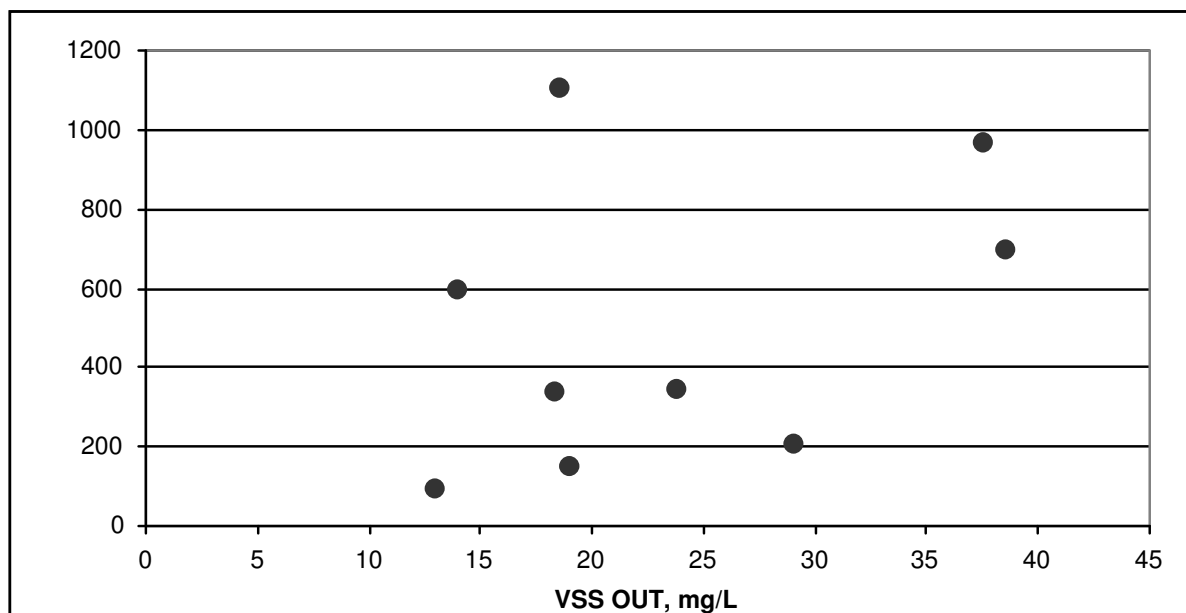
C) Digester Evaluations

The concentrated algal sludge that is produced through the CEP process has several potential applications. One promising concept is to digest the algal material in an anaerobic digester, thereby converting a portion to methane, which in a full-scale CEP installation could be combusted in a generator to produce electrical power to defray a portion of the total operating costs. Also, the exhaust from this combustion would contain large amounts of carbon dioxide, which could be used in the Algal Treatment Zone to stimulate algal productivity. Detailed studies of this concept were conducted in 4.0 liter laboratory and 500 gallon field anaerobic digesters located at Clemson University. The initial results indicated that harvested algal sludge alone was a poor substrate for methane production. However, laboratory studies indicated that the performance of the digesters could be radically improved by adjusting the C/N ratio of the digester feed with shredded waste paper.

Studies were conducted to improve the digestibility and fuel recovery from algal biomass by combining the algal sludge with waste paper. The algal sludge evaluated ranged from 2% volatile solids content, harvested with metal salts induced flocculation, to 6% volatile solids content when harvested using filter feeding fish as a harvesting and concentrating technique. The species of dominant algae in the sludge varied by season, but the bulk of algal biomass was composed of *Scenedesmus sp*. or *Chlorella sp.* We investigated the effects of loading the digester with varying amounts of paper at a fixed amount of algal sludge loading. The composition at each loading rate for co-digestion at 10 days hydraulic retention time is given in Table 7. Co-digestion of these mixtures resulted in the maximum methane production rate at a loading rate of 5 g VS/L-day and 60% paper fraction (C/N=22.6/1). Under these conditions, the digester was capable of producing 1,600 to 1,800 cm^3 of CH_4 per day per liter of reactor volume (as compared to only 200 cm^3 day from algal sludge alone). Volatile fatty acids (VFA)

concentration increased from 1,300 mg/L (at 2 g algal VS/L-day) to a maximum of 6,200 mg/L (at 5 g paper + 2 g algal VS/L-day). TAN concentration was observed to decrease from 589 mg/L to less than 200 mg/L.

Table 7. Observed methane production, VFA, TAN, and C/N ratio in co-digestion of algal sludge and waste paper at a fixed algal loading rate of 2 g VS/L-day and 10 days HRT.

Loading g VS/L d	Paper g VS/L d	Algal sludge g VS/L day	CH_4 ml/L d	VFA mg/L	TAN mg/L	Alk×1000 mg/L	C/N	5N NaOH ml/L day
2	0	2	180±34	1305±147	589±86	4.6±0.35	6.7	0
3	1	2	823±16	3780±458	541±2	4.7±0.2	13.3	0
4	2	2	1170±75	3912±1290	524±24	5.4±0	18	0
5	3	2	1607±17	5220±855	396±8	4.3±0.4	22.6	0.25
6	4	2	856±40	6228±685	175±7	4.3±0.3	27.2	1.5

In these studies, anaerobic co-digestion of algal sludge with waste paper produced a dramatic, eight-fold increase in the methane production rate. Methane yield was seen to increase from less than 100 cm^3/g VS loaded to 325 cm^3/g VS loaded (Figure 32). The digestion process appears to benefit from the combination of reduced ammonia concentration under low C/N ratio feedstock loading (under algae loading alone) and added ammonia under high C/N feeding (under paper alone feeding) and increased cellulase activity under conditions of the combined algae/paper loading. The observed optimal C/N ratio ranged from 18/1 at a loading rate of 4 g VS/L-day (50% algae/paper mix) to 22.6/1 at a loading rate of 5 g/L-day (60% paper). Cellulase activity was observed to increase 3-fold with paper addition.

Figure 32. Observed methane production vs loading rate at fixed algal loading of 2 g VS/L and varying paper fractions at 10 days HRT.
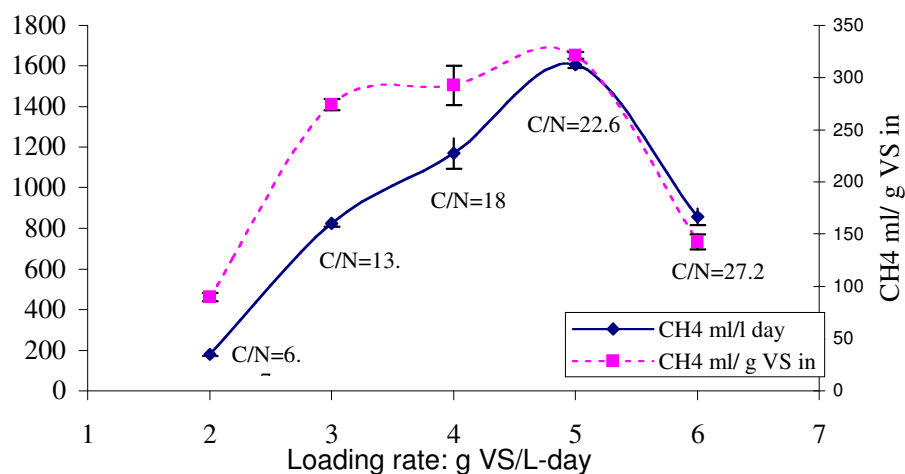
Field studies with a 500 gallon prototype reactor demonstrated the need for mechanical mixing of the combined paper /algal slurry to insure proper digester performance. We loaded and operated the field digester with adjusted algal sludge under mixed conditions to determine the optimal parameters for large-scale operation. These results were encouraging and if they prove to be applicable in commercial-scale CEP applications, could significantly affect the cost of CEP treatment.

Gas production rates for the anaerobic digesters at Clemson University suggested that an intermittently-mixed digester (twice per day) would be more efficient than a continuously-mixed digester using mixed algal feedstock. The digestion results observed in this study agree with other research reports in the literature suggesting that a closer interaction between microbial consortia (by less mixing) was important to the anaerobic digestion process, and that continuously mixing did not result in increased methane production rates in the case of Algal/paper digestion.

Task 8. Utilize the resulting data to provide economic projections of the capital and operating costs involved in a full-scale implementation of the Controlled Eutrophication Process for the entire Salton Sea.

Using our measurements of the efficiency of CEP water treatment technology in treating water taken from the Whitewater River, we extrapolated the results to full-scale commercial operation. In order to estimate the economics of CEP systems as accurately as possible, records were maintained of the capital and operating involved in the pilot-scale CEP systems and were used in our studies concerning the costs of a Demonstration-Scale project. The results of the economic analyses are combined with the results for the Demonstration-Scale analyses and are presented on the following pages (Task 9).